Dr Dunn

RESEARCH AND EXTRAMURAL STUDIES

PLANS AND OBJECTIVES FOR 1982

C82-06880

CONFIDENTIAL

T. S. OSDENE

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

CONFIDENTIAL

To: Dr. T. S. Osdene

Date: November 4, 1981

From: J. L. Charles

Subject: Plans and Objectives for 1982--Biochemical Research Division

The Plans and Objectives prepared by the individual project leaders in the Biochemical Research Division are attached. Each project leader prepared his/her report after receiving input from his colleagues and meeting with the manager, technical coordinator, and other project leaders so that project plans are consistent with overall plans and objectives of the division.

The objectives are:

1. Develop an integrated program for control of insects which infest stored tobacco, processed filler and finished cigarettes.

2. Develop methods for the collection, isolation, identification and quantitation of tobacco and cigarette components which affect the *in vivo* and/or *in vitro** bioactivity of smoke products.

3. Establish a matrix or battery of *in vitro* bioassays for the evaluation of the biological effects of new cigarette products, additives, and smoke products.

4. Conduct research investigations to determine the factors responsible for the biological activity of smoke products in each assay in the battery.

An overview of the plans in their logical progression is presented below.

*_in vivo_ means biological studies conducted in whole animals (mammals).

in vitro means biological studies conducted in culture.

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CHARGE NUMBER 1101--ENTOMOLOGICAL RESEARCH

Cigarette beetle physiological studies will be continued. The continuing trend to eliminate the use of highly toxic pesticides as control agents dictates a continued effort in this area. Research in this area will consist of: (1) the effect of temperature and relative humidity on beetle growth and survival; (2) the evaluation of azadirachtin from neem seeds as an insect feeding inhibitor; (3) the evaluation of serricornin, a synthetic sex pheromone, as a trap bait for primary and manufacturing areas; (4) investigation of prism projected rays as a light attractant; (5) studies of the adult beetle flight and response behaviors; (6) development of a screening technique for inhibitors of the larval midgut symbiont, and (7) the study of NALED (a low toxicity organophosphate) as a sex pheromone beetle trap toxicant.

The research program associated with the "commercial trial" of KABAT®, 5% methoprene in ethanol, will continue. A study of bright tobacco treated with 5 ppm methoprene was reinitiated in November, 1980 and will continue through November, 1982. The Maury Street, Chesapeake and Cabarrus warehouses storing only KABAT® treated tobacco will be monitored by checking beetle trap counts. We will continue to support the stemmeries in KABAT® application throughout PM USA and PMI facilities as requested.

Upon request, we will continue to provide consultation and technical services on insect control problems for other Departments within Philip Morris Incorporated. These services will consist of on-site inspections for existing or potential infestation of stored or processed tobacco, providing insects and insect counts for control tests and providing updated information on state and federal regulations pertaining to the use of pesticides. Long range monitoring studies involving steam or steam-vacuum insect killing efficacy checks will be continued.

CHARGE NUMBER 6910--CONDENSATE PREPARATION, PROCESSING, AND PYROLYSIS STUDIES

The collection and processing of cigarette smoke condensate and other smoke products is crucial since these products are starting materials for most of the other projects in the division. An outside study beginning in the first quarter of 1982 will require Elmenhorst cold trapped condensate, preparation of acetone solutions of processed condensate, and quality assurance determinations of nicotine and water in condensate. This is a high priority program requiring a continuing effort throughout the year.

Mainstream smoke will continue to be collected for *in vitro* and chemical testing. Various methods used for collection of condensate and/or smoke volatiles include impaction trapping, solvent filled impaction trapping, Elmenhorst cold trapping, Cambridge filter pad and volatiles or gas phase collection techniques.

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Sidestream cigarette smoke (SS) collection methods will continue to be developed and assume increasing importance. Current methods involve smoking a single cigarette at a time and methods will be investigated to allow simultaneous smoking of four cigarettes in a sidestream smoke chamber for small scale *in vitro* and chemical testing. To support sidestream smoke condensate fractionation studies, we will initiate studies on methods to collect sidestream smoke from a 30-port Borgwaldt smoking machine.

As with mainstream smoke, methods for collecting sidestream smoke will include impaction trapping, vapor phase cold trapping, solvent filled impaction trapping, and Cambridge pad collection. These methods will be used to support chemical and *in vitro* studies. We will continue to evaluate Cambridge pad collected SS directly extracted with dimethylsulfoxide (DMSO) in screw capped vials to facilitate direct MS-SS comparisons on the same cigarette and avoid extraction of pads in a fritted funnel.

Trapping of SS volatile and nonvolatile nitrosamines in gas washing bottles will continue to be investigated. Methods will be devised in collaboration with 6908 to introduce nitrogen into SS at varied flow rates in an effort to reduce artifactual formation of volatile nitrosamines.

The small Mellen furnace with a quartz pyrolysis tube allows pyrolysis of filler at temperatures up to 900°C. This method will continue to be used in the investigation of the effects of pyrolysis conditions on the *in vitro* biological activity produced from pyrolysis of a number of fillers and blends. Pyrolyzate base fractions will be prepared for *Salmonella*/microsome testing and chemical profiling. Filler treatment with potassium salts or nicotine prior to pyrolysis and the effects on chemical profiling and *in vitro* activity will be investigated.

Nitrogen-free LTF-5E control sheet and LTF-5E plus various additives will be prepared on an "as needed" basis. The sheet material will be shredded for filler, made into cigarettes and smoked for chemical and biological studies.

CHARGE NUMBER 6908--CHEMICAL STUDIES OF CONDENSATE

Chemical studies of condensate will continue to focus on the isolation, identification, and quantitation of compounds thought to be relevant to the *in vitro* and *in vivo* biological activity of smoke products. This effort requires a continual monitoring of the literature related to biologically active smoke components.

Both volatile and nonvolatile nitrosamines will be determined in sidestream smoke with methods development in conjunction with 6910. Filler and MS smoke nonvolatile nitrosamines will be determined on a series of model cigarettes with the aim of establishing a data base. Nitrosamine determination will continue on a request basis for Project 6900.

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Burley CSC base fraction studies, in conjunction with 6906 will continue. Major emphasis will be directed toward HPLC separation of the (*Salmonella*) active LH-20 fractions from the toluene/50% aqueous methanol partition step carried out on burley base fraction pot residue. Subfractions with significant activity will be further separated with isolation/identification of active compounds as a goal.

Evidence for the presence of Glu-P-1/Glu-P-2 (pyrolysis products of glutamic acid) has been obtained on an LH-20 fraction of burley base fraction and isolation and NMR confirmation will be attempted.

The feasibility of concentrating *Salmonella*/microsome activity of burley base fraction and subfractions by preparative GPC will be studied.

The effect of nicotine on *in vitro* activity will be studied in collaboration with 6902, 6904, 6906, and 6910. Nicotine at several levels will be added directly to the pot residue from burley and bright base fractions and tested by *in vitro* assays to determine if a direct effect of nicotine can be demonstrated. Similar studies will be carried out on CSC and acid/neutral fractions from burley and bright tobacco cigarettes. A comparative study will then be conducted on bright and burley cigarettes with varying levels of added nicotine to determine the relationship, if any, between nicotine, nicotine pyrolysis products and *in vitro* biological activity. In addition a nicotine aza-arene study will be initiated with nicotine added to LTF-5E followed by an isolation of nicotine derived (or not) aza-arene fraction. Using gc, gc/ms, and the *Salmonella*/microsome assay, an attempt will be made to correlate activity with individual components. Identification of these active components will follow.

Work will begin on a HPLC/*in vitro* interface with 6902 and 6906 with the goal of determining specific activity of each peak in an HPLC chromatogram using the *Salmonella*/microsome or the 8-azaguanine forward mutation assay.

Interactive effects in which inhibitory compounds are masking the *in vitro* effects of various fractions have been demonstrated and will be investigated further.

Research proposals will be developed for microsome-independent activity (MIA), electrophiles-glutathione (GSH) interactions and other biologically active compounds--cell--smoke interactions.

Sidestream studies involving nitrosamines, fractionation and chemical characterization of SS fractions will continue or be initiated.

The method for HPLC analysis of carbonyls in VP will be reestablished and updated to be used in a collaborative study with 6904 using the L5178Y assay.

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A collaborative study with 6902 on the D_4 yeast strain and activity of acid/neutral and base fractions will be completed. A PAH fraction procedure and capillary gc analysis will be utilized to study a selected number of cigarettes for comparison of *in vivo* activity, *in vitro* activity and PAH profiles.

Work on the thermal decomposition of duvatriene diols and tobacco diterpenes will be completed shortly.

Testing of base fractions from MS and SS smoke derived from RL base web with added KOAc will be completed. The fractions will be analyzed for 2AC and results correlated with *Salmonella*/microsome assay using LTF plus amino acids or protein as the starting material.

After initial installation and training on the VG MM-16 gc/ms/ds, evaluation of various packed and/or capillary columns for base fraction analysis will begin. Evaluation of a fast atom bombardment ion source for nonvolatiles and consideration of desorption chemical ionization to enhance volatility will be pursued.

A Chromatotron has been ordered for use in base fractionation studies of CSC.

CHARGE NUMBER 6906--BIOLOGICAL EFFECTS OF SMOKE

The majority of the *Salmonella*/microsome mutation assay studies will involve a continuing collaborative interaction with 6900, 6908, and 6910 project personnel. These studies are directed towards refining our understanding of the determinants of CSC activity and the reader is referred to the 6906 Plans and Objectives for a detailed treatment.

Studies involved with assay standardization and quality assurance will include transition from the Sigma to the DEC system, preparation of a new TA98 frozen ampoule stock culture and purchase and complete evaluation of a new batch of Aroclor®-1254 induced rat liver microsomes. Aroclor® induced hamster liver microsomes will also be evaluated to broaden our metabolic activation capability. A determined effort will be made to complete the interfacing of the automatic colony counter with the main computer (with CAD personnel) to improve our ability to handle an increased workload.

A research proposal will be written to approach the evaluation of a number of filler and construction parameters on CSC activity (effect of dilution, rod weight, RTD, firmness, cuts per inch, *etc.*, on coal temperature and IT CSC activity).

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In a collaborative study with 6902 and 6904, a research proposal will be developed to address the question of assay sensitivity and specificity when known mutagens are added to CSC and later when known mutagens are added to filler and the resulting smoke condensate is tested in the *Salmonella*/microsome assay (and other assays). This is a crucial study which will aid us in interpreting results obtained on biological activity testing of additives plus tobacco.

CHARGE NUMBER 6904--BIOCHEMICAL METHODS DEVELOPMENT AND UTILIZATION

This project is involved with the development of short-term *in vitro* mammalian cell assays for evaluation of potential biological activity of cigarette smoke products or potential additives.

A difficulty with the L5178Y mouse lymphoma assay has been the inability to obtain a negative response in the assay in our laboratory. Pure chemical compounds known to produce no bioactivity *in vitro* or *in vivo* will be tested. If this is unsuccessful, a redefinition of a negative response will be in order. The MS and SS activity of LTF-5E (nitrogen-free) will be evaluated in the L5178Y assay and filler composition will be altered to manipulate activity.

The V79 Chinese Hamster lung cell assay is being developed using a mutation endpoint. Dose-response relationships with a known direct acting mutagen has been established and will be replicated. A mutagen requiring metabolic activation will be studied next followed by reproducibility studies and additional classes of compounds. This assay is attractive since it has the potential for multi-endpoints. In that regard, efforts are underway to establish the "promotion assay" endpoint of V79 which is based on the apparent inhibition of metabolic cooperation by promoters. The assay will be established with the potent promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Dose-response studies with other pure compounds (known promoters and negative compounds) will follow.

CHARGE NUMBER 6902--BIOCHEMICAL SPECIAL INVESTIGATIONS

The yeast mitotic gene conversion assay using strain D_4 is a continuing effort in this project. This assay suffers from an apparent lack of sensitivity to CSC and fractions. Good dose-response curves have been obtained with CSC but frequently the activity does not exceed two times background. A number of acid/neutral and base fractions are being studied with the specific goal of acquiring sufficient data to make an intelligent decision whether or not to proceed with this assay. Plans for 1982 are dependent on the outcome of that evaluation. Strain D_7 has been added to our collection and will be evaluated in the mitotic gene conversion endpoint for utility in additive testing.

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In 1982, we will establish the *S. typhimurium* forward mutation assay based on 8-azaguanine resistance. This assay is attractive because the genetic target for forward mutation is much larger than the reverse mutation of the Ames reverse mutation assay. Also, the forward mutational event (normal cell is made sick) mimics the real life situation better than reverse mutation (sick cell made normal). So, the combination of decreased specificity coupled with the forward mutation should increase our capability for detecting a mutagen. The type of mutational event (base substitution or frame-shift mutation) can be studied with the Ames tester strains. This assay will require a mini-validation study as part of its establishment.

We also expect to establish the Sister Chromatid Exchange assay in 1982 as a sensitive indicator of chromosomal damage and for monitoring of DNA repair. We will use the Chinese hamster V79 cells for this assay and further utilize the multi-endpoint capability of that cell line. Definitive plans are dependent on filling an open requisition with a qualified person.

The *Tradescantia* stamen hair assay will be studied for feasibility with regard to its potential for use in monitoring SS biological activity in dilute atmospheres.

Finally, the emphasis on new assay development will shift. Previously assays were developed for measuring the biological activity of cigarette smoke condensate; a most complex task certain to require extensive development before useful results were obtained. Only then was an assay used to evaluate potential additives. In this writer's opinion, Philip Morris would be better served if an assay were established with pure compounds and then utilized immediately for testing additives. In this way, the advantage of the assay to PM is immediately apparent. Then one could proceed to the much more complex task of elucidating the biological activity of cigarette smoke condensate and fractions. It is this approach which will establish a *battery* of short-term *in vitro* assays in the shortest possible time.

nwp

cc: Dr. R. N. Ferguson
Dr. M. A. Manzelli
Dr. T. T. Yu
Dr. R. W. McCuen
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Dr. R. A. Pages

Attachments: six

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: . Mr. J. L. Charles

Date: October 20, 1981

From: . M. A. Manzelli

Subject: . PLANS AND OBJECTIVES FOR 1982 (CHARGE NUMBER: 1101)

The single objective of the Entomological Research Group is to reduce insect damage to stored and processed tobaccos.

Specific objectives and plans for the year 1982 are as follows:

OBJECTIVES

I. CIGARETTE BEETLE PHYSIOLOGICAL STUDIES

A. To continue the study of beetle growth and survival at various combinations of temperature and R. H.

B. To continue to evaluate feeding inhibitors, when candidate compounds are available.

C. To continue to evaluate the synthetic sex pheromone, serricornin, in the field and to initiate tests in the primary and manufacturing areas under low population densities. Additional laboratory studies will also be made.

D. The adult beetle light attractant study will be continued in the laboratory utilizing prism projected rays.

E. The adult beetle flight and response behaviors will be studied further.

F. To continue the development of a screening technique for the inhibitors of the larval midgut symbiont.

G. The use of NALED, an organic phosphate pesticide (of low toxicity) will be studied as a sex pheromone beetle trap toxicant.

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II. KABAT® USAGE

A. To monitor the Maury Street Chesapeake and Cabarrus warehouses storing only KABAT® treated tobacco. This will be done by checking the beetle trap counts.

B. To continue to assist the stemmeries with their analytical problems.

C. To continue the 5 ppm KABAT® stemmery/warehouse test through November, 1982.

III. CONSULTING AND TECHNICAL SERVICES TO AREAS OUTSIDE OF R & D

A. On a request basis, assistance will be given to areas experiencing insect control problems.

B. All long range monitoring programs involving steam or steam-vacuum cycle efficacy checks will be continued.

PLANS AND TIME ALLOCATION

I. CIGARETTE BEETLE PHYSIOLOGICAL STUDIES

We need more knowledge of the cigarette beetle's physiology and of its response to both chemical and physical stimuli in order to devise improved methods for its control. These studies will require a maximum of 11.5 man-days a week.

II. KABAT® USAGE

The support given to accumulating efficacy data on the 10 ppm methoprene treatments will be greatly decreased this coming year. However, support will be continued in developing and recording efficacy data from the 5 ppm methoprene applications. These studies will require 3.5 man-days per week.

III. CONSULTING AND TECHNICAL SERVICES TO AREAS OUTSIDE R & D

As in the past, these services will continue to consist of on-site inspections for either existing or potential infestations of stored or processed tobaccos; of providing insects and counts for insect control tests of various types; and in providing updated information on state and federal regulations pertaining to the use of pesticides. This service will require 5.0 man-days per week.

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M E Manzelli

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ALLOCATION OF MAN-DAYS PER WEEK

(TOTAL = 20 MAN-DAYS)

ACTIVITY	M. F. MINOR	J. S. LONG	R. M. LEHMAN	M. A. MANZELLI	TOTALS
Cigarette Beetle Physiological Studies	3.5	3.5	3.5	1.0	11.5
KABAT® Usage	1.0	0.5	1.0	1.0	3.5
Consulting and Technical Services to PM Areas	0.5	1.0	0.5	3.0	5.0
TOTAL MAN-DAYS	5.0	5.0	5.0	5.0	20.0

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Mr. J. L. Charles Date: October 29, 1981

From: T. Yu

Subject: Project Charge Number 6902 (Biochemical Special Investigations)--
Plans and Objectives for 1982

I. OBJECTIVES

a) To investigate the feasibility of new assay systems for the evaluation of cigarette smoke products.

b) To establish and to apply short-term *in vitro* assays to evaluate potential activities of:

1) new cigarette additives, and

2) cigarette smoke products

c) To determine the component(s) responsible for cigarette smoke product activity in each assay.

The yeast mitotic gene conversion assay will be a continuing effort from the former Project 6906. New short-term assays in the areas of bacterial mutagenesis (*Salmonella typhimurium*-forward mutation based on 8-azaguanine resistance) and mammalian cell cytogenetics (sister chromatid exchange) will be added to the project. In addition, a feasibility study of using a plant system (*Tradescantia* stamen hair mutation assay) to evaluate the potential activity of cigarette smoke will also be carried out in 1982.

We are actively recruiting two staff members for Project 6902: a scientist and a research scientist. Both will be experienced individuals who can work independently on the planned assay systems. The research scientist will be involved in the sister chromatid exchange assay. In this regard, the time schedule for this assay will be planned after the research scientist joins the project.

II. PLANS

A. Yeast Mitotic Gene Conversion Assay

1. Introduction

This assay has been a part of a battery of short-term assays for the past several years. *Saccharomyces cerevisiae* strain D_4 has been used as the tester strain. To date, the activity of a variety of samples have been

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determined in this assay system. The results showed that all CSC samples tested generally exhibited a dose-response effect and that the assay is able to distinguish activities among various types of smoke products derived from different cigarettes. One drawback of these results is the fact that the average overall sensitivity of the assay is not very high. For example, the activity of the majority of the samples tested did not show a two-fold increase over that of the background control, which is the generally accepted criterion for a test compound to be active in short-term *in vitro* assays.

2. Plans

1) Strain D_4 (Fourth Quarter, 1981 and First Quarter, 1982)

a. Continue to study the cigarette smoke product activity in close collaboration with Project 6908 personnel as in the past. Experiments are currently carried out to test burley: CSC, acid, base, and neutral fractions. The results of these experiments will be used as guidelines to determine the directions of work involved with strain D_4. Upon completion of this work, a special seminar will be given by L. H. Thompson to the Manager, Technical Coordinator, and the Project Leaders of the division to make a thorough evaluation of the yeast mitotic gene conversion assay using strain D_4. A definitive decision whether to use strain D_4 for future work should be reached no later than the first quarter, 1982.

2) Strain D_7 (First Quarter, 1982 and continuing)

Strain D_7 has been added to our culture collection in 1980. This strain was constructed in a manner that three genetic effects may be monitored simultaneously in the same strain. These effects are: mitotic gene conversion, mitotic recombinations and reverse mutation. Initial experiments will be carried out to establish the D_7 assay to monitor mitotic gene conversion with control compounds. Current plans involving work with strain D_7 are primarily concerned with testing potential new cigarette additives. Testing of CSC and/or other smoke products would be conducted only as time permits.

B. *S. typhimurium* Forward Mutation Assay Based on 8-Azaguanine Resistance

1. Introduction

The forward mutation assay employs one tester strain, T667. It is designed to measure the ability of a test agent to produce mutant cells in a wild type cell population. The resultant mutant cells are selected by cultivating the treated cell population on growth medium containing 8-azaguanine (8AG), which supports growth of the mutant cells, but not the wild type cells. The assay can be conducted both in the presence and absence of metabolic activation (±S9). Because it is a forward mutation, the target size of the genetic material responsible for the mutational event(s) is larger compared with reverse mutation. Furthermore, it should not exhibit

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mutagen specificity. Theoretically, it should be more, and/or at least as sensitive as the standard *Salmonella*/microsome assay which measures reverse mutational events.

The forward mutation assay has not been widely validated. Literature reports showed that the forward mutation assay measured in one tester strain is equisensitive to the *Salmonella*/microsome assay using five tester strains simultaneously.

The forward mutation assay will be used to test cigarette smoke products and potential new cigarette additives.

2. Plans

Work concerning the forward mutation assay has been planned in three phases:

Phase 1: Assay Establishment and Validation (First Quarter, 1982)

Initially, a visit will be made to the outside laboratory where the assay was developed. Two goals will be accomplished during the visit. (1) To become familiar with all aspects of the mutation assay and to acquire the necessary stock cultures. (2) To learn and to become familiar with the procedures involved in interfacing the microassay version of the forward mutation test to evaluate activity of HPLC fractions, particularly with respect to automation. Subsequent to the visit, the assay will be established at PM using positive and negative control compounds in the presence and absence of metabolic activation (±S9).

After assay establishment, effort will be directed toward building a data base for assay validation. Fifteen to 20 compounds from different classes of chemical will be selected and evaluated for their response in the assay. Preferably, these will be paired compounds consisting of active and inactive counterparts from the same chemical class.

Phase 2: Evaluation of Smoke Products Derived from Selected Cigarettes (Second Quarter and Continuing)

This phase of work will be coordinated closely with Project 6906 and 6908 personnel.

1) Cigarettes selected for investigation will be Kentucky reference cigarette 2R1, burley, a low tar filler and RL. The activity of CSCs and acid, base and neutral fractions derived from these CSCs will be evaluated. Subfractions of the active fractions will be further evaluated. The purpose of these studies will be to establish a data base which can be compared with that from the standard *Salmonella*/microsome assay.

2) Interface forward mutation assay with HPLC fractions. Burley cigarettes will be used for these investigations. Fractions that are active in the forward mutation assay will subsequently

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be tested in *Salmonella*/microsome assay using all five strains. This is done with the attempt to characterize the mutation specificity, *i. e.*, whether the detected activity is due to base-pair substitution or frame-shift mutation. The results generated from these biological studies will be used to guide further work in chemical separation of fractions and/or subfractions.

Phase 3: Automation--to be determined

C. Sister Chromatid Exchange (SCE) Assay

1. Introduction

Cigarette smoke condensates have been reported to induce SCE in test systems containing no exogenous metabolic activating enzymes. In our plans, the SCE assay will be used to evaluate the activity of potential new cigarette additives and cigarette smoke products. In addition, the influence of these substances on cell replication kinetics and mitotic index will also be investigated in selected cases.

2. Plans

SCE assay will be conducted in V79 Chinese hamster lung cells. This cell line was acquired by Project 6904 personnel recently and mutation assays using this cell line are currently being carried out.

Plans for SCE assay include:

1) Assay establishment using positive and negative control compounds both in the presence and in the absence of metabolic activation (±S9).

2) Assay utilization as a part of a battery of short-term assay in routine evaluation of potential new cigarette additives, and, later, cigarette smoke products.

3) Assay validation by evaluating the activity of selected cigarette CSCs and derived fractions. Cigarette selected will be 2R1, burley, a low tar filler and RL.

D. *Tradescantia* Stamen Hair Mutation Assay

This assay system is potentially suitable to study the activity of sidestream smoke. The feasibility for conducting this assay at PM should be investigated. The feasibility study will involve literature search, contact with and visits to outside laboratories and a complete assessment of the assay system.

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SUMMARY FOR PLANS AND OBJECTIVES FOR 1982

	Assay/Activity	Time
A.	Yeast Mitotic Gene Conversion Assay	
	1. Strain D_4	4th Quarter, 1981 and 1st Quarter, 1982
	2. Strain D_7	1st Quarter, 1982 and continuing
B.	*S. typhimurium* Forward Mutation Based on 8-Azaguanine Resistance	
	1. Assay establishment and validation	1st Quarter, 1982
	2. Evaluation of smoke products from selected cigarettes	2d Quarter and continuing
	3. Automation	To be determined
C.	Sister Chromatid Exchange Assay	To be determined
	1. Assay establishment	
	2. Evaluation of potential new cigarette additives	
	3. Assay validation using cigarette smoke condensates and derived fractions	
D.	*Tradescantia* Stamen Hair Mutation Assay: A Feasibility Study	

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Mr. J. L. Charles Date: October 29, 1981

From: R. W. McCuen

Subject: Project Charge Number 6904 (Biochemical Methods Development and Utilization)--Plans and Objectives for 1982

The objectives of this project are similar to those of Project Charge Number 6906 before it was split. These are:

a) to develop short-term mammalian cell *in vitro* assays to evaluate the potential biological effects of cigarette smoke products or potential additives.

b) to conduct research investigations designed to understand the nature of cigarette smoke product activity in each *in vitro* assay.

The mammalian cell bioassays we will develop and/or use to accomplish these objectives are described below.

1. L5178Y Mouse Lymphoma Assay

Investigators: D. Ayers (Associate Scientist B) and M. Penn (Scientist) functioning as a team.

The two major objectives, plans to attain these objectives, and projected time frames in which the work will be done are as follows:

A. Observe a negative response(s) in the $TK^{+/-}$ mutation assay in our laboratories. We will test pure chemical compounds KNOWN to produce no bioactivity (other than toxicity) *in vitro* and/or *in vivo*. Such compounds as acetone, pyrene, ethylene glycol, sucrose, and gibberellic acid will be tested. [A revision of our SOP definition of a positive and/or negative response in this assay may be necessary at the conclusion of this work.]

First Quarter and Continuing

B. Elucidate some of the filler/smoke parameters associated with the activities of various tobacco or nontobacco smoke products in the $TK^{+/-}$ mutation assay. This will involve personnel of Project Charge Numbers 6908 and 6910. We will characterize (with and without microsomal activation, dose-response curves, *etc.*) MS (IT CSC, VP and SIT) and SS bioactivity from the nitrogen-free high organic, low ash NTSM--LTF-5E cigarette.

First and Second Quarters

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The above results will be documented in a special report. An attempt will then be made to change the basal activity level of the various LTF-5E products stated above by altering the filler composition. Such alterations *might* include the addition of nicotine, urea, or specific amino acids.

Third Quarter and Continuing

[NOTE: As part of this objective and as a carry over from some work done in 1981, we plan to test the VP of selected cigarettes for their L5178Y bioactivity in relation to their contents of certain carbonyl type compounds (acetaldehyde, formaldehyde, acrolein, diacetyl, and methyl vinyl ketone). Thus, we will be in a position to determine if a quantitative relationship exists.]

Second Quarter

C. From conversations with J. L. Charles, we expect to receive some requests for testing CSCs and/or potential cigarette additives in 1982. This work will be conducted on a high priority basis.

Continuing

2. V79 Chinese Hamster Lung Cell Assay

The two major objectives, plans to attain these objectives, projected time frames in which the work will be done and the principal investigator are as follows.

A. ESTABLISH the mutational endpoint (HGPRT marker) of this cell system in our laboratories. The work will be conducted by L. Weissbecker (Research Scientist). [Eventually, it is our goal to replace the L5178Y/TK+/- mutation assay with the more versatile, multi-endpoint V79 bioassay.] Initially, we plan to learn general cell handling techniques, cell characteristics, *etc.* Ethyl methanesulfonate (EMS) will be tested as a nonactivation positive control using 6-thioguanine (6-TG) as a selective agent in order to establish dose-response curves, assay reproducibility, and variability.

First Quarter

Next, we plan to test a positive activation control, 7-12-dimethylbenz(a)anthracene (DMBA), using the same selective agent with microsomal activation in order to establish dose-response curves, assay reproducibility, and variability.

Second and Third Quarters

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The above three quarters of work will be documented in a special report. Additional pure agents representing chemical classes other than polycyclic aromatics (DMBA) will be tested with microsomal activation as described above in an attempt to extend the second and third quarter results. The *completion* of this work will ESTABLISH this assay system in our laboratories and be in a position to use it for potential cigarette additive testing in 1983.

Fourth Quarter and Continuing

B. ESTABLISH the "promoter assay" endpoint of this cell system in our laboratories. The work will be conducted by R. McCuen (Associate Senior Scientist). [This is part of the multi-endpoint capabilities of this cell system as alluded to above.] This assay is based on the following observations. Wild-type Chinese hamster V79 cells (6-TG^S) reduce the recovery of 6-TG^Y cells when co-cultured at high cell densities in the presence of 6-TG. This reduction has been shown to occur because of intercellular communication (metabolic cooperation). Metabolic cooperation is inhibited by most known promoters, thus rescuing the 6-TG^Y cells. This assay is not based on the interaction of an active agent with DNA. Some evidence has been presented in the literature which shows that this assay can discriminate between tumor promoters possessing various degrees of *in vivo* potency. Initially, we plan to learn general cell handling techniques, cell characteristics, *etc.* Several spontaneously- and/or EMS-induced HGPRT^- (6-TG^Y) mutants will be obtained, pooled, and a cell stock prepared.

First and Second Quarters

The promoter assay will be conducted using HGPRT competent and HGPRT deficient V79 cells and the known promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA) in an effort to establish dose-response curves, assay reproducibility and variability.

Third Quarter

Finally, we hope to test other positive (butylated hydroxytoluene and phenobarbital) and negative (phorbol) pure chemicals in this system to determine dose-response curves, assay reproducibility and variability. If successful, the V79 HGPRT promoter assay would be ESTABLISHED. These results would be documented in a special report.

Fourth Quarter and Continuing

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SUMMARY OF PLANS AND OBJECTIVES FOR PROJECT CHARGE NUMBER 6904 FOR 1982

Assay/Activity	Time
1. L5178Y/TK$^{+/-}$ Mutation Assay	
A. Negative control compound	first quarter and continuing
B. Determinants of cigarette activity	
LTF-5E	first quarter and continuing
VP/carbonyl studies	second quarter and continuing
Additive and CSC testing	upon request
2. V79 Chinese Hamster Lung Cell Assay	
A. Establish HGPRT mutational endpoint	
Test EMS without S9	first quarter
Test DMBA with S9	second and third quarters
Document results	fourth quarter
Test other classes of agents with S9	
B. Establish HGPRT promoter assay endpoint	
Learn cell handling techniques Obtain stock of HGPRT$^-$ cells	first and second quarters
Conduct assay with TPA	third quarter
Test other positive and negative controls Document results	fourth quarter

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Mr. J. L. Charles Date: October 29, 1981

From: Karen Rapp Sherwood

Subject: Project Charge Number 6906 (Biological Effects of Smoke)--Plans and Goals for 1982

1. INTRODUCTION

The objectives of this project remain essentially unchanged.

a) To utilize the *Salmonella*/microsome assay in a battery of short-term *in vitro* assays to evaluate the potential effects of cigarette smoke products.

b) To conduct tests on potential new cigarette products or additives upon request and to assist in the evaluation and interpretation of the results obtained.

c) To conduct research investigations designed to generate an understanding of the nature and control of cigarette smoke product activity in the *Salmonella*/microsome assay.

2. PLANS AND GOALS FOR 1982--*SALMONELLA*/MICROSOME MUTATION ASSAY

The majority of these studies will involve continuing collaborative interactions with various 6908 and 6910 project personnel which are directed toward refining our understanding of the determinants of CSC activity in conjunction with other similar studies by projects 6902 and 6904, and testing on request from 6900 project personnel. We anticipate a significant increase in testing for both of these areas, especially requests for testing CSCs and/or potential cigarette additives by 6900 project personnel.

A. Assay Standardization and Quality Assurance

In order to keep the *Salmonella*/microsome assay up and running, several studies are necessary in or by the first quarter, 1982. These include: 1) a complete transition from the Sigma to the DEC system in order to retain all of our current computer capabilities for handling and storing our data; 2) preparation of a new TA98 frozen ampoule stock culture for use as inoculum in routine testing; and, 3) purchase and complete evaluation of a new batch of Aroclor®-1254 induced rat liver microsomes according to our SOP (for microsome evaluation) for use in routine testing. Since Aroclor®-induced hamster liver microsomes are, or soon will be available from project 6904, we would also like to evaluate them (at the same time that we evaluate the rat liver microsomes) for a preliminary data base

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should we need to use them for testing/screening potential cigarette additives, fractions, *etc.*, in the future. By the end of the second or third quarter, 1982, the interface of the automatic colony counter directly to the main computer (with the assistance and services of CAD) will be completed in order to help us to handle our data more efficiently with our increased work load; and the "*Salmonella*/Microsome Assay Methods Manual" which documents our standardized operating procedures in this laboratory will be issued. As soon as the new *Salmonella typhimurium* tester strains which are reported to detect additional classes of biologically active compounds are available from Ames' laboratory, we are planning to obtain them for our bank of tester strains. Based on the literature on these strains and our own project priorities, we may evaluate them for their potential use in our laboratory. In addition, we will continue our efforts to carefully monitor the assay results on positive and negative control compounds, viable counts, and markers on a routine basis.

B. Additive and CSC Testing

Based on information provided by 6900 project personnel (R. A. Pages, R. D. Carpenter, and J. E. John), we are expecting an increase in requests for testing CSCs and/or potential cigarette additives in 1982 over those from 1981. As in the past, this area of work will continue to be conducted promptly and on a high priority basis.

C. Sidestream *versus* Mainstream Activity (with 6910)

Our major interest in determining the activity of sidestream *versus* mainstream CSC (or TPM), in this and other *in vitro* assays, is to try to relate any observed differences in activity to differences in CSC composition and/or to the different temperature/pyrosynthesis, *etc.*, conditions that are recognized for cigarette filler that is burned statically *versus* dynamically. The results of the work conducted to date show: 1) sidestream CSC or TPM is less active than mainstream CSC or TPM; 2) the activity of sidestream basic fraction is less than that of the mainstream basic fraction; 3) mainstream TPM is less active than mainstream IT CSC; 4) the activity of sidestream IT CSC from statically burned cigarettes is the same as that from cigarettes burned in the normal manner (58 sec. puff interval); and, 5) from preliminary 2R1 data, there is evidence that the sidestream TPM from statically burned cigarettes is more active than the sidestream IT CSC from statically burned, or dynamically smoked cigarettes. The experiments thus far have been restricted to the use of the 2R1 KRC, RCB, burley, bright, and RL reference cigarettes and some control RL base web and K^+ salt added samples on RL base web (KOAc and KCl). These studies will continue in 1982. Due to the high water content of sidestream samples, some further work will be done to better profile the dose range in order to try to increase our ability to measure sidestream activity in the assay, and to determine the best dose-response range for testing. Once the collection procedure for sidestream smoke, the TPM extraction procedure, and the method of preparing TPM stock solutions in DMSO is standardized, we plan to retest the five model cigarettes to compare sidestream *versus* mainstream TPM collected from the same cigarettes under static/dynamic burn

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conditions. It is hoped that we will then be able to answer some of the questions on the absolute and relative sidestream *versus* mainstream activity. The results of these studies will further dictate what direction we will then take; *e. g.*, expand testing to other cigarette types; testing of additional sidestream fractions (acid/neutral, base, and 2AC/H/NH, *etc.*); and trying to relate activity to chemical composition.

D. Cigarette Filler Pyrolysis (with 6910 and 6908)

This area of investigation will continue to be expanded during 1982. On a continuing basis, we are seeking to define what relationships may exist between pyrolyzate and CSC activity and composition with a view toward ultimately being able to generate samples for testing when cigarette fabrication is difficult or impossible and/or to evaluate the major precursors of CSC activity. It may also be possible that the pyrolyzate work could lead to setting up an additional model system for studying sidestream activity. So far, the parameters involved in generating pyrolyzate samples that have been studied in 1981 are: large batch *versus* small batch; carrier gas flow rates; activity *versus* temperature from 300°-900°C in 100°C increments; air *versus* nitrogen; helium *versus* nitrogen; and 20 minute *versus* 12 minute pyrolysis time. Base fractions from 2R1 pyrolyzate were also tested. In 1982, we will continue to study the activity of pyrolyzate *versus* atmosphere and temperature for model cigarette filler types and dry *versus* conditioned filler; base fractions: pyrolyzate *versus* IT CSC activity (also, acid/neutral fractions eventually); and pyrolysis of salt effect filler samples. Testing of pyrolyzates from bright, burley, and 1:1 blended samples initiated in the fourth quarter, 1981, will continue in 1982. A study to determine the effect of a low temperature filler sweep before pyrolysis and/or sweeping with heated gas on pyrolyzate activity is planned in the third or fourth quarter, 1982, followed by investigations on preheated filler. Additional testing in 1982 may include the pyrolysis of LTF + trp for the quantitation of carbolines (*versus* activity), and an investigation into fractional pyrolysis (collecting fractions as a function of temperature) in conjunction with personnel in the Analytical Division involved with studies of evolved gas analysis.

E. Filler Precursors of CSC Activity (with 6908 and 6910)

Research in this area is an outgrowth of our longstanding interest in trying to evaluate tobacco fractions to see which, if any, may be major nitrogen-containing precursors of IT CSC activity. In 1981, our efforts were concentrated in the following areas: 1) the effects of salts added to filler; 2) model cigarette additives; and 3) the pyrolysis of cigarette filler (discussed previously in Section D). In 1982, we plan to continue: 1) the salt effects studies (KOAc + RL base web) by measuring recovered CSC activity in base fractions, and 2) the model cigarette additive studies with sugars, amino acids, and proteins on LTF filler.

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F. HPLC: Testing of Active Fractions (with 6908 and 6902)

In order to further our efforts to isolate and identify the active components in burley CSC base fractions, we plan to initiate a study in the first quarter, 1982, to test the activity of peaks (representing less than one mg of material) generated by the HPLC of active base fractions of interest. It has been reported that a similar method has been set up and is being used in conjunction with the *Salmonella typhimurium* 8-azaguanine forward mutation assay at M.I.T. Currently, it is planned that a member of project 6902 will visit this laboratory at M.I.T. in order to bring back the 8-azaguanine assay and the methodology for HPLC testing. Members of 6908 will take this methodology (modifying it if necessary for applications in this laboratory) and generate the samples for us to test in the standard *Salmonella*/microsome assay.

G. Denitrated RL Studies

Studies on the effect of various processing parameters and additives on the IT CSC activities of autogenous denitrated RL samples were initiated in 1981, upon the request of R. D. Carpenter and will be continued in 1982. This testing will be done on a prompt and high priority basis.

H. Water Expansion and Related Studies (with G. Keritsis)

In previous years, we have tested bright tobacco expanded by all of the current expansion processes. In 1981, we obtained control and water expanded (steam) uncased bright samples whose IT CSCs showed no significant difference in activity when tested in the *Salmonella*/microsome assay. Because we had never tested expanded burley tobacco, we requested control and water expanded cased and uncased burley machine made cigarettes for testing in the assay. Since the samples that we received were all diluted (between 10 to 15 percent), the samples were tested with and without dilution so that our interpretation of the results was not further complicated by this additional variable. The results showed: 1) the activity of uncased burley was significantly more active than cased burley, with or without dilution; 2) the activity of the diluted samples was significantly different--uncased control>uncased expanded>cased control>cased expanded; and, 3) the activity of the undiluted samples only showed the casing effect and did not show any significant difference in activity between the uncased (control and expanded) samples (like the bright studies), or between the cased (control and expanded) samples. These results were significant because it was the first time that we had seen a casing effect on CSC activity, and we are planning to continue studies in this area in 1982. These results also pointed to the fact that we need to look at some of the cigarette parameters which could explain these results based on differences in smoking characteristics such as coal temperature.

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We plan to write a research proposal in the second quarter, 1982, offering an experimental design for evaluation of the effect of a number of filler and construction parameters on CSC activity, (*e.g.*, effect of dilution, coal temperature, rod weight, RTD, firmness, cuts per inch, *etc.*, on IT CSC activity), so that we can request that the appropriate cigarettes be made, and we can begin testing by the fourth quarter, 1982.

I. CSC + Mutagen Study (with 6902 and 6904)

To begin with, a joint research proposal aimed at a broad approach to this problem will be written by the end of the first quarter, 1982. Once this proposal has been worked out and the mutagens/CSCs to be used are selected, we plan to initiate this study in the *Salmonella*/microsome assay by testing mutagens added to CSC (first quarter, 1982). This study will then continue and will be further expanded to CSCs from filler that has been treated with a known mutagen prior to smoking. Our efforts in this area are dictated by the high priority of this study and will be blocked off into a logical sequence of steps so that testing can be achieved as add-ons to our routine testing schedule.

J. CSC Fractions and Model Compounds (with 6908)

Work on isolating and identifying the active components in burley CSC basic fractions (LH-20 fractions from toluene/aqueous methanol extracts) will continue in 1982, along with the model compound studies (Glu-P-1/Glu-P-2; IQ). Beginning in the second or third quarter, we will test fractions from a new preparative gel permeation chromatography procedure to determine how the activity separates and what the differences, if any, there are in comparison to the LH-20 procedure. Testing of acid/neutral fractions to look for any interactive effects (isolate/identify possible active compounds and/or inhibitors) should proceed in the second quarter, 1982. Studies to look at some biochemical interactions, *e. g.*, microsome independent activity of IT *versus* ECT fractions from high nitrate cigarettes in tester strain TA100, are planned in the second or third quarter, 1982. In addition, the testing of PAH fractions from model cigarettes should be completed by the end of the first quarter, 1982.

K. Effect(s) of Nicotine on Activity (with 6908)

We plan to initiate this study in the first quarter, 1982. The study as currently outlined will proceed in three defined phases: 1) to determine the direct effect of nicotine on bright and burley base fraction activity; 2) to determine the direct effect of nicotine on CSC and acid/neutral fraction activity; and, 3) to determine the effect of nicotine in tobacco on the activity of CSC fractions. We anticipate that this high priority study will be completed in the third quarter, 1982. Beginning sometime in the second or third quarter, 1982, we will also begin testing LTF + nicotine samples in conjunction with the proposed aza-arene study (6908).

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3. SUMMARY--PLANS AND GOALS FOR 1982

Salmonella/Microsome Mutation Assay	Time
A. Assay Standardization and Quality Assurance	
-Preparation of new TA98 frozen ampoule culture stock for routine inoculum	First quarter
-Obtain/evaluate new batch of Aroclor®-induced rat liver microsomes for routine testing	First quarter
-Evaluate hamster liver microsomes	First quarter
-Make the transition from the Sigma to the DEC system	Fourth quarter, 1981
-Finish the interface of the automatic colony counter to the main computer	Second - Third quarter
-Issue Methods Manual	Second quarter
-Monitor assay response	Continuing
-Test new strains	When available
B. Additive and CSC Testing	Upon request by 6900 project personnel
C. Sidestream *versus* Mainstream Studies	
-Standardize method of sidestream TPM collection, extraction, and preparation of stock solutions in DMSO	First quarter
-Retest 2R1, RCB, burley, bright, and RL reference cigarettes	Second - Third quarter
-Test other cigarette types	Third - Fourth quarter
-Test sidestream fractions: acid/neutral, base, 2AC/H/NH	Second - Third quarter
D. Cigarette Filler Pyrolysis	
-Pyrolyzate *versus* atmosphere and temperature for model cigarette types and filler types	First quarter
-Base fraction: Pyrolyzate *versus* IT CSC activity; also, acid/neutral studies	Second quarter and continuing
-Effect of low temperature sweep and/or sweeping with heated gas on pyrolyzate activity	Third - Fourth quarter

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D.	Cigarette Filler Pyrolysis *(continued)*	
	-Fractional pyrolysis	Fourth quarter
	-Pyrolysis of salt effect samples	First quarter
	-Pyrolysis of bright, burley, and 1:1 blend	Fourth quarter, 1981 and continuing
	-Investigations of preheated filler	Third - Fourth quarter
	-Dry filler *versus* conditioned filler	First quarter
E.	Filler Precursors of CSC Activity	
	-Salt effects (KOAc + RL base web): base fractions	Continuing
	-LTF studies: LTF + sugars, amino acids, and/or proteins	Third - Fourth quarter
F.	HPLC: Testing of Active Fractions	First quarter and continuing
G.	Denitrated RL IT CSC Studies: testing of autogenous denitration samples; additives; and processing parameters	Continuing--by request (6900)
H.	Water Expansion Studies	
	-Cased *versus* uncased burley	Continuing
	-Effect of filler and construction parameters on coal temperature and CSC activity	
	--Research Proposal	Second quarter
	--Sample Evaluation	Fourth quarter
I.	CSC + Mutagen Study	
	-Research Proposal	First quarter
	-Mutagen added to CSC	First quarter
	-Mutagen added to filler → test CSC	Second quarter and continuing

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J.	CSC Fractions and Model Compounds	
	-Burley CSC base fraction	
	--LH-20 fractions from toluene/aq. MeOH extracts	Continuing
	--Preparative GPC and/or Chromatotron procedure	Second - Third quarter
	--Model compounds: Glu-P-1/Glu-P-2; IQ	Second - Third quarter
	-Test acid/neutral fractions for interactive effects: isolate/identify possible active compounds and/or inhibitors	Second quarter and continuing
	-Biochemical Interactions: microsome independent activity of IT *versus* ECT fractions from high nitrate cigarettes (TA100)	Second - Third quarter
	-PAH fractions from model cigarettes	First quarter
K.	Effect(s) of Nicotine on Activity	
	-Direct effect on base fraction activity	First quarter
	-Direct effect on CSC and acid/neutral fraction activity	First quarter
	-Effect of nicotine in tobacco on the activity of fractions	Second - Third quarter
	-Nicotine aza-arene study (LTF + nicotine)	Second - Third quarter and continuing

Karen Rapp Sherwood

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PHILIP MORRIS INCORPORATED

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: . Mr. J. L. Charles Date: October 30, 1981

From: . A. H. Warfield

Subject: . Plans and Objectives for 1982--Chemical Studies of Condensate (Charge Number: 6908)

I. INTRODUCTION

As presently constituted due to the recent changes implemented in the Biochemical Research Division, our principal areas of activity are as follows:

a) Isolate, identify, and quantitate smoke components with emphasis on those substances with *in vitro* or *in vivo* activity.

b) Relate active products to filler precursors to understand the origin of *in vitro* assay activity.

c) Maintain chemical or analytical capabilities necessary for ongoing and collaborative studies.

Since our objectives are largely relative to maintaining our competitive position in the marketplace, we continually monitor potentially significant areas in the literature and at technical meetings. In addition to our stated plans, we also must respond when necessary to developments in the literature perceived as important from a biochemical standpoint.

II. RESEARCH PLANS

A. Nitrosamines

1. Samples from Project 6900

Both volatile and nonvolatile nitrosamines will be determined on a request basis, using procedures developed previously. These samples may include filler, MS, or SS smoke, and all involve gc/tea as the final analytical step.

2. Sidestream Smoke (with 6910)

Depending on developments in trapping methods, further work will be done on application of the methodology for volatile and nonvolatile nitrosamines in SS smoke. A number of routine and research nitrosamine determinations will be made.

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3. Model Cigarettes

In order to provide a data base for nonvolatile nitrosamines, filler and MS smoke from a series of model cigarettes will be analyzed by our standard methods. The data obtained will be used to augment the data on volatile nitrosamines previously obtained on the same models.

B. Burley CSC Base Fractions

1. LH-20 Fractions (with 6906)

The major emphasis will be directed toward HPLC separation of the (*Salmonella*) active LH-20 fractions from the toluene/50% aqueous methanol partition step carried out on X6D3IM base fraction pot residue. Subfractions showing significant activity will be further separated with the goal of isolation/identification of active compounds.

2. Model Compounds

a) Glu-P-1/Glu-P-2

Spectral and chromatographic evidence has been obtained for the presence of these compounds in an LH-20 fraction from X6D3IM base fraction. Further cleanup followed by gc/ms/SIM is planned. Eventual isolation and NMR confirmation will be attempted if possible. Some quantitative work may be done if justified from the results obtained.

b) IQ

A very small sample of 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ), a mutagen isolated from cooked beef and fish, has been obtained. An attempt to determine whether this compound can be detected in burley base fraction is planned.

3. Preparative GPC Procedure (with 6906)

Four columns packed with Biobeads SX-12 have been set up and will be tested for their ability to concentrate the *Salmonella*/microsome activity of burley base fraction and subfractions. The effect of solvent on the type of separation obtained will be studied. Ultimately, the method may be utilized in the burley base fraction isolation effort.

C. Effect of Nicotine on *In vitro* Activity (with 6902, 6904, 6906, and 6910)

1. Direct Effect on Base Fraction Activity

Nicotine will be added to pot residue obtained from burley and bright CSC base fractions, at several levels. The samples will be tested in *Salmonella*, and possibly in yeast, and L5178Y to establish whether or not a dose response for nicotine can be observed.

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2. Direct Effect on CSC and Acid/Neutral Activity

Studies similar to that carried out on base fraction will be repeated on CSC and acid/neutral fractions from burley and bright tobacco cigarettes.

3. Effect of Nicotine in Filler on Activity of Fractions

Various levels of nicotine will be added to bright and burley tobacco, cigarettes made and smoked, and acid/neutral and base fractions obtained. The base fractions will be distilled yielding pot residues. CSC and fractions will be tested to determine whether differences in the *in vitro* activity attributable to a nicotine effect can be detected. Depending on results, fractionation may be carried out to determine which compounds are responsible for increased activity. A related approach to this problem using radiolabeled nicotine will be in progress concurrently in the Chemical Research Division. Sufficient collaboration will be maintained to avoid redundancy in effort.

4. Nicotine Aza-arene Study

Plans for this study were described in a recent Research Proposal. A CSC base fraction from LTF containing a nicotine salt will be compared with the corresponding fraction from LTF control, using gc, gc/ms, and the *Salmonella*/microsome assay. Procedures for isolating a basic aza-arene fraction will be evaluated. An attempt will be made to correlate activity with individual peaks on a gc trace. Identification of active compounds will follow. If time permits, a study of the effect of nitrate and trapping method may be initiated.

D. HPLC/*In Vitro* Activity Interface (with 6902, 6906)

1. HPLC/*Salmonella*/Microsome Assay

A method for determining the approximate specific activity of each peak in an HPLC chromatogram using relatively small samples will be developed. The use of an RI detector to avoid the necessity of weighing the eluting fractions will be attempted. The resulting HPLC/activity profile should increase efficiency in isolation/identification of active compounds, and eventually result in reduced sample loads in 6906.

2. HPLC/8-azaguanine Forward Mutation Assay

When the above assay is established by 6902, the technology can be transferred to this assay to allow detection of a broader range of activity, and still further reductions in sample loads in Project 6906.

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E. Interactive Effects in *Salmonella* (with 6906)

Various fractions of CSC show low activity in the *Salmonella*/microsome assay, but in effect may have significant activity which is masked by inhibitory compounds in the fractions. Demonstration of the effect in certain fractions has occurred recently when LH-20/methanol chromatography was followed by *Salmonella* testing.

Fractions under consideration are acid/neutrals, ether eluate from Al_2O_3 chromatography of base fractions, and early LH-20 fractions from toluene and aqueous MeOH extracts of X6D3IM pot residue. Appropriate isolation/identification efforts will be made depending on results.

F. Biochemical Interactions

1. Microsome-Independent Activity (with 6906)

An attempt will be made to look for microsome-independent activity in the *Salmonella* assay in CSC and fractions from high nitrate cigarettes, where ECT are used. A research proposal will be written on this subject, which will include the available internal literature and a literature search, since a number of related studies on nitro compounds in materials such as diesel exhaust have appeared.

2. Electrophiles-GSH Interactions

A Research Proposal on this topic will be written after conducting a literature search.

3. Other Biologically Active Compounds--Cell--Smoke Interactions

A literature search is in progress, which will be followed by a Research Proposal.

G. Sidestream Studies (with 6906 and 6910)

1. Nitrosamines

Volatile and nonvolatile nitrosamines will be determined on sidestream samples generated by Project 6910 (see Section II.A.2.).

2. Fractionation

Acid/neutral and base fractions will be prepared from SS smoke when received from Project 6910. These samples will be submitted for *Salmonella*/microsome assay.

3. Chemical Characterization of SS Smoke Fractions

Depending on sample size and availability, efforts will be made using gc/ms, HPLC, *etc.*, to obtain information on the composition of sidestream smoke.

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4. 2AC/H/NH Analysis

Base fractions from SS smoke will be analyzed by our procedure for 2AC, harman, and norharman. The results will be correlated with *Salmonella*/microsome data.

5. Carbonyls in SS-VP by HPLC

Modification of the carbonyl procedure will be made to allow collection of SS-VP and conversion to DNPH derivatives for HPLC analysis. The initial DMSO solution of VP may be submitted for L5178Y and/or yeast assay.

H. Carbonyls/L5178Y/Collaborative Study (with 6904, 6910)

1. Model Compounds

Necessary purification or procurement of model compounds for study of L5178Y activity will be carried out.

2. Carbonyls/HPLC Analysis

The method for HPLC analysis of carbonyls in VP will be reestablished and updated. The method will then be used in conjunction with LTF studies proposed by Project 6904.

I. D_4 Yeast--CSC Fraction Activity (with 6902)

1. Optimize Dose Ranges

Acid/neutral and base fraction dose ranges will be established in order to obtain more meaningful data and allow accountabilities to be calculated.

2. Test Fractions

Depending on the results obtained above, further fractions will be prepared and tested to allow a better understanding of how this assay responds with respect to other assays.

J. PAH Fraction (with 6906)

1. Model Cigarettes

When the HP5880A gc is coupled to the HP computer, a series of model cigarettes will be subjected to the recently developed PAH procedure, and pattern recognition data correlated with *Salmonella*/microsome activity and EBA. The method will also be applied to high and low nitrate filler, and to CSC trapped by IT and ECT, to yield data on changes occurring due to nitrate or trapping method.

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K. Terpene Studies

1. Thermal Decomposition of Duvatriene Diols

An internal report summarizing on-column duvatriene-1,3-diol thermolysis is planned, as well as a possible external publication. The final stages of the work should be completed in 1981.

2. Tobacco Diterpenes

An internal report on previously completed studies of the antifungal properties of tobacco diterpenes will be prepared, and an external publication is planned as well.

L. Filler Precursors of Activity

1. Salt Effects (with 6906, 6910)

Preparation and testing of base fractions from MS and SS smoke derived from RL base web containing added KOAc will be completed shortly. Additionally, these fractions will be analyzed for 2AC, and the results correlated with *Salmonella*/microsome data. GC/MS profiling will be used in an attempt to understand the salt effects.

2. LTF Studies

Initial planning is now under way on a study designed to identify additives which will modify the *Salmonella*/microsome activity of CSC or base fractions from LTF containing nitrogen compounds such as amino acids or proteins. This will incorporate effects of cation ratios, *etc.* A Research Proposal will be written on this subject.

M. Chromatography/Spectroscopy

1. GC/MS Studies

After initial installation and training on the VG MM-16 gc/ms/ds, evaluation of various packed and/or capillary columns for base fraction analysis will begin. Evaluation of a fast atom bombardment ion source for nonvolatiles, and consideration of desorption chemical ionization to enhance sample volatility will be pursued. GC/MS of samples submitted as part of ongoing studies will continue.

2. Chromatotron

A Chromatotron has been ordered for use in connection with fractionation of CSC. After initial familiarization, this instrument will be used for at least some of the base fractionation studies now in progress, as well as other studies being planned.

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III. SUMMARY OF PLANS FOR 1982

Activity	Time
A. Nitrosamines	
1. Samples from Project 6900	Continuing
2. Sidestream Smoke	
3. Model Cigarettes	
B. Burley CSC Base Fractions	
1. LH-20 Fractions	Continuing
2. Model Compounds	
a) Glu-P-1/Glu-P-2	Continuing, Complete by second quarter
b) IQ	Third quarter
3. Preparative GPC	Initiate first quarter Testing second and third quarters
C. Effect of Nicotine on *In vitro* Activity	
1. Direct effect on base fraction activity	First quarter
2. Direct effect on acid/neutral activity	First quarter
3. Effect of nicotine in filler on activity in fractions	Second and third quarters
4. Nicotine aza-arene study	Initiate first quarter
D. HPLC/*In vitro* Activity Interface	
1. HPLC/*Salmonella*/microsome assay	First quarter
2. HPLC/8-azaguanine forward mutation assay	Third or fourth quarters
E. Interactive Effects in *Salmonella*	Initiate second quarter

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	Activity	Time
F.	Biochemical Interactions	
	1. Microsome-independent activity Research proposal Test samples	 First quarter Second - third quarters
	2. Electrophiles-GSH interactions (Research proposal)	First quarter
	3. Other biologically active compounds--cells--smoke interactions (Research proposal)	First quarter
G.	Sidestream Studies	
	1. Nitrosamines	
	2. Fractionation	Second or third quarters
	3. Chemical characterization	Fourth quarter
	4. 2AC/H/NH analysis	Second or third quarters
	5. Carbonyls in SS-VP	Fourth quarter
H.	Carbonyls/L5178Y/Collaborative study	
	1. Model compounds	Second quarter
	2. Carbonyls/HPLC analysis	Fourth quarter
I.	D_4 Yeast--CSC Fraction Activity	First quarter
J.	PAH Fraction	First quarter
K.	Terpene Studies	First quarter
L.	Filler Precursors of Activity	
	1. Salt effects	Continuing
	2. LTF Studies Research proposal Testing	 First quarter Third or fourth quarter

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	Activity	Time
M.	Chromatography/Spectroscopy	
1.	GC/MS Studies	Continuing
2.	Chromatotron	Initiate first quarter

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Mr. J. L. Charles Date: November 2, 1981

From: R. D. Hellams

Subject: Charge Number 6910 (Condensate Preparation, Processing, and Pyrolysis Studies) Plans and Objectives for 1982

I. INTRODUCTION

The objectives of this newly formed project (October 1, 1981) were formerly a part of Charge Number 6908.[1] The principal areas of activity for this project are summarized in the following goals:

a) Collect and process cigarette smoke condensate for *in vivo* testing.

b) Collect and process mainstream cigarette smoke by appropriate methods for *in vitro* and chemical testing.

c) Study methods for smoking and collection of sidestream cigarette smoke for *in vitro* and chemical testing.

d) Develop and study pyrolysis conditions on the pyrolysis of varied cigarette fillers to aid in understanding *in vitro* assay activity.

e) Prepare, smoke, and/or pyrolyze varied LTF formulations for use in a collaborative study of precursor-smoke product relationship on *in vitro* or *in vivo* assay activity.

II. *IN VIVO* STUDY

Our major responsibility for the coming year is to supply smoke condensate for an *in vivo* test beginning the first quarter of 1982.

This test will consist of four cigarette types to be smoked. The condensate will be trapped in Elmenhorst cold traps[2] and processed to prepare acetone solutions of the condensate for outside testing.

The following plans and objectives for 1982 for this project are contingent due to the time and personnel available to first accomplish the above major responsibility.

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III. MAINSTREAM CIGARETTE SMOKE (with 6900, 6902, 6904, 6906, and 6908)

Trapping Methods for *in vitro* and Chemical Testing

Mainstream cigarette smoke will continue to be collected for *in vitro* and chemical testing. Various trapping methods either alone or in combination have been used for the collection of cigarette smoke condensate and/or smoke volatiles.

1. The Impaction Trap

The impaction trap[3] has been the major trapping technique used to collect smoke condensate for *in vitro* and chemical testing in our laboratory. This trapping technique is ideal for numerous small batch smokings for *in vitro* testing. The smoke condensate sample is taken up directly in DMSO for testing without further handling.

2. The Solvent Filled Impaction Trap

An impaction trap containing a solvent such as DMSO will be used to trap mainstream smoke volatiles for special *in vitro* tests such as the L5178Y thymidine kinase (TK) mutation assay. Other such uses of this technique will be the trapping of nicotine in an acid solution for the volatile phase exiting the dry impaction trap during the collection of mainstream and sidestream smoke.

3. The Elmenhorst Cold Trap

The Elmenhorst cold trap has been the principal collection method for collecting large quantities of cigarette smoke condensate to be used for *in vivo* tests. This collection system will continue to be used for special smoke condensate tests such as acids, bases, and neutral fractionation studies for chemical and/or *in vitro* testing. It will be used to collect smoke condensate from high nitrate cigarettes in a comparative study of the chemistry of this condensate *versus* that of condensate collected in an impaction trap.

4. The Cambridge Filter Pad

The Cambridge filter pad will continue to be used in the collection of mainstream smoke in the sidestream smoke studies as well as for small tests to check the delivery of a new cigarette.

5. Volatiles and/or Gas Phase Collection

Collection of volatile phase and gas phase cigarette smoke continues to be of interest in the areas of chemical analysis and *in vitro* assay activity of such compounds as carbonyls. Profiling studies of volatile phase and/or gas phase of cigarette smoke by appropriate methods (gc, lc, gc/ms) will be pursued as time permits.

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IV. SIDESTREAM CIGARETTE SMOKE (with 6902, 6904, 6906, and 6908)

A. Smoke Chamber Design Study

The increasing importance of sidestream smoke studies point to the need of a faster method for smoking and collecting sidestream cigarette smoke. The present method of smoking one cigarette at a time in a sidestream smoke chamber is time-consuming. An ideal initial improvement for collecting sidestream smoke would be the ability to smoke at least four cigarettes at one time in a sidestream smoke chamber for small scale *in vitro* and chemical testing.

B. Large Scale Collection of Sidestream Cigarette Smoke

An even greater need is the large scale collection of sidestream smoke from a 30-port Borgwaldt smoking machine for use in sidestream smoke condensate fractionation studies. Other areas of interest would be the collection of the volatile phase and the gas phase of sidestream cigarette smoke. The initial attempt at a large scale collection method for sidestream smoke will focus on the particulate phase.

C. Collection for *in vitro* Testing and Chemical Testing

Appropriate collection methods will be utilized for sidestream smoke collection whether for *in vitro* testing or for chemical testing.

Sidestream impaction trapped condensate samples for a limited number cigarette types will be tested in the *Salmonella*/microsome assay.

Additional chemical fractionation studies of impaction trapped sidestream smoke for varied cigarette types are planned. Of particular interest is base fraction *Salmonella*/microsome activity and MS/SS ratios for components such as 2-amino-α-carboline.

Vapor phase sidestream smoke collected in a cold trap or in an impaction trap containing a solvent will be collected for chemical profiling studies (gc, gc/ms) as time permits.

Cambridge pad collected sidestream TPM will be collected and compared in the *Salmonella*/microsome assay to Cambridge pad collected mainstream smoke for the same cigarette(s). Initial prior tests of these pads placed directly in DMSO in a screw capped vial to reduce water absorption from the atmosphere have been successful as a test procedure. This procedure will continue to be evaluated as an efficient procedure to eliminate DMSO extraction of the pads in a fritted funnel. This would also reduce equipment cleanup time.

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D. Nitrosamines in Sidestream Smoke

Initial experiments were successful for the collection of sidestream volatile and nonvolatile nitrosamines. Two gas washing bottles in series containing pH 4.5 citrate buffer with ascorbic acid--as used by Project 6908 for volatile and nonvolatile nitrosamines in mainstream smoke--are connected to the exit of the presently used sidestream smoke chamber.[4] Development work with 6908 is planned in the area of introducing nitrogen into the sidestream smoke stream at varied flow rates to determine the effect of reducing artifactual formation of volatile nitrosamines. Also, the technique will be used to determine if there is an effect on the nonvolatile tobacco-specific nitrosamines in the more inert atmosphere.

E. Collaborative Effort with Charge Number 4009 on High Priority Samples

During this year several cigarettes with special wrappers to reduce sidestream smoke were tested by the sidestream smoking-testing procedure we are presently using. Due to the high demand of time on our projected goals for 1982, this type sample for sidestream smoke "analysis" will need to be of a high priority nature as determined by the Biochemical Research Division Manager and/or Technical Coordinator.

IV. PYROLYSIS STUDIES (with 6906 and 6908)

The small Mellen furnace has been used successfully during the current year for many pyrolysis studies. The simpler designed quartz pyrolysis tube has allowed the pyrolysis of filler at higher temperatures (up to 900°C *versus* previous maximum of 650°C). Also, the filler sample size has been reduced from 5 grams as used with the large Mellen furnace to 2 grams with the smaller oven.

Additional studies are planned which include the pyrolysis of varied fillers and blends, the effect on activity of varied oxygen atmospheres *versus* the nitrogen and air atmospheres presently being studied.

The preparation of pyrolyzate base fractions from selected fillers are planned for *in vitro Salmonella*/microsome assay testing and chemical profiling. Pre-treatment of filler as with potassium salts or nicotine prior to pyrolysis and alternate pyrolysis procedures are planned.

Pyrolyzate chemistry such as the gc, gc/ms study of the volatiles not trapped in the DMSO used to collect the pyrolyzate for *in vitro* testing will be undertaken as time permits.

V. LTF PREPARATION STUDIES (with 6904, 6906, and 6908)

The preparation of LTF-5E control sheet material or control LTF with various additives will be used to support varied research studies. The sheet material will be prepared on an "as needed" basis. The sheet material will be shredded for filler and made into cigarettes and smoked for *in vitro* tests and chemical tests, or as filler for pyrolysis studies.

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VI. SUMMARY OF PLANS FOR 1982

Activity	Timetable
A. *In Vivo* Study	Initiate first quarter
1. Four cigarette types to be smoked	
2. Elmenhorst cold trapped	
B. Mainstream Cigarette Smoke	
1. Collection methods for *in vitro* and chemical studies	
a. Impaction trap	Ongoing
(1) Normal smoking procedure for trapping CSC for	
(a) *in vitro* tests	
(b) chemical analysis	
b. Elmenhorst cold trap	
(1) Special tests	
(a) nitrate addition to filler	Second - third quarter
(2) Large quantities of CSC	
(a) chemical fractionation studies	On request
c. Solvent filled impaction trap	
(1) Special tests	
(a) L5178Y	Ongoing
(b) nicotine	Second - third quarter
d. Cambridge pad	
(1) *in vitro* assay	Second - third quarter

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Activity	Timetable
C. Sidestream Cigarette Smoke	
1. Smoke chamber design study	Initiate second - third quarter
a. Chamber to smoke at least four cigarettes	
2. Collection for *in vitro* testing	Initiate first quarter and continuing
a. Impaction trap	
b. Elmenhorst cold trap	
c. Solvent filled impaction trap	
d. Cambridge pad	
3. Collection for chemical testing	Third - fourth quarter
a. Impaction trap	
b. Elmenhorst cold trap	
4. Large scale collection for sidestream condensate	Second - third quarter
a. Collection from 30-port Borgwaldt smoking machine--design study	
5. Nitrosamines	First - second quarter
D. Pyrolysis Studies	
1. Water in filler effect on pyrolysis, activity	First quarter
a. 2R1, bright, burley, or 50/50 blend	
b. 300-900°C temperature range	
c. Nitrogen atmosphere	
2. Varied oxygen in nitrogen atmosphere	First quarter
a. One or more levels of oxygen	
b. 2R1, bright, burley, or 50/50 blend	
c. 300-900°C temperature range	

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Activity	Timetable
3. RL base web + K^+ salts	First quarter
a. RL base web control	
b. RL base web + KCl	
c. RL base web + KOAc	
(1) atmosphere to be determined	
(2) 300-900°C temperature range	
4. Pyrolysis base fractionation studies	Second quarter
a. Bright	
b. Burley	
c. Blend	
d. RL + K^+ salts	
5. Research proposal for pyrolysis of pre-treated fillers and alternate pyrolysis procedures	Third quarter
6. Alternate filler types including LTF	Third quarter
7. Pyrolyzate chemistry	Fourth quarter
a. Volatiles	
(1) gc, gc/ms	
b. Other fractions	
(1) Acids, bases, neutrals	
(a) gc, gc/ms	
(b) HPLC	
(c) other	
E. LTF Preparation Studies	Ongoing

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REFERENCES:

1. Ferguson, R. N. 6908 Annual Report--Smoke Condensate Studies. Annual Report 81-124; 1981 June 3.

2. Elmenhorst, H. Eine neus Kältefalle zur Gewinnung grosser Mengen von Tabakrauchkondensat. Beiträge zur Tabakforschung 3:101-107. 1965 Mai.

3. Hellams, R. D. Development and evaluation of an impaction trap for the collection of cigarette smoke condensate. Completion Report 80-256; 1980 October 21.

4. Hellams, R. D. Comparison of sidestream smoke of control X6DOBOI (R & D 3346) and experimental X6DOBOM (R & D 3350) cigarettes. Memo to Ms. B. Goodman. 1981 March 12.

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Ralph D. Hellams

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Dr. T. S. Osdene Date: November 5, 1981

From: E. B. Sanders

Subject: 1982 Plans and Objectives - Chemical Research Division

The objectives for the Chemical Research Divison during 1982 are as follows:

A. Precursor-Product Studies

The majority of the effort in this area will be devoted to a thorough study of the contribution of nicotine to the formation of aza-arenes. Less extensive studies will be directed towards 1) an attempt to determine the nitrogenous precursor(s) of the nitroso group in volatile nitrosamines; 2) the smoke chemistry of rutin; and 3) the determination of the contribution of the various blend components to mainstream smoke of low and ultra-low delivery cigarettes. All of these projects will utilize labelled precursors and all, except for the last study, will attempt to identify precursors which lead to undesirable products with the hope of ultimately learning to eliminate such products thereby leading to improved products. The last study represents a rational approach to blending in low delivery cigarettes.

A very different type of precursor-product study is represented by our proposed work with Amadori compounds. We anticipate that a detailed study of the products formed from the thermalization of a number of these compounds (via either pyrolysis or on smoking) may lead to an improvement of the cooked flavor system.

B. Isolation and/or Synthesis of Labelled Compounds

This work will be carried out to provide labelled compounds necessary for both precursor-product and filter efficiency studies. Compounds to be made include ^{14}C-nicotine, ^{14}C-alkylpyrazines, ^{14}C-menthol, ^{14}C-rutin, and $^{15}NO_3^-$ and $^{15}NO_2^-$.

C. Investigations of Thermal Mechanisms

The ultimate goal of precursor-product studies

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is to identify the precursors of either undesirable or desirable products and then to control their formation. Although considerable work has been done in the past involving the "identify" portion of these studies, little has been done with regard to "control." As a consequence we plan to initiate a detailed study of the reaction of nitrite (assuming that we first experimentally confirm nitrite as a direct precursor of volatile nitrosamines in a cigarette) with secondary amines as a function of several reaction variables such as temperature, pH, etc. Based on the results of these studies we would hope to be able to rationally modify the chemistry taking place in a cigarette to reduce the formation of volatile nitrosamines.

D. Transfer of Inorganics to Smoke

These studies will continue with most of the emphasis being placed on the transfer of calcium utilizing ^{45}Ca labelled tobacco. Calcium transfer to smoke has previously been shown to be atypical, and it is hoped to discover the causes of this anomolous behavior.

E. Low Level Laboratory

Construction of the low level laboratory should be completed in 1982. Initial work projected will be environmental monitoring of tobacco and tobacco by-products.

F. Development of New Instrumental Methods

Improved methodology is required to improve our ability to identify products formed in precursor-product relationships. Specific methods which will be investigated include 1) liquid chromatography-mass spectrometry, 2) low level counting and, 3) high resolution capillary gas chromatography coupled to mass spectrometry.

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G. Flavor-Release Research

We will continue to pursue the synthesis of flavor-release compounds, with most of the activity

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being devoted to carbohydrate-based systems. In addition to the synthetic program, however, it is also planned to explore smoke chemistry problems using flavor-release compounds. Flavor-release compounds represent a unique opportunity to study smoke chemistry in that they are well characterized non-volatile compounds which give limited numbers of products on pyrolysis. In addition pyrolysis products are generally a function of temperature. Consequently, a detailed investigation of the behavior of flavor-release compounds on cigarettes should provide concrete information on the rates of heat transfer in a burning cigarette. As a consequence, not only does the area of flavor-release chemistry provide a potential source of new products, it also will greatly contribute to our understanding of smoke chemistry.

H. Selective Filtration of Flavorants

We are currently studying the transfer of tetramethylpyrazine to mainstream smoke using gas chromatographic techniques, and will shortly initiate a similar study with menthol utilizing radiochemical analytical techniques. Both of these compounds are of interest in that significant transfer from filler to filter takes place. As a consequence we will be able to determine the amount of flavorant transferring from the filter to mainstream smoke as well as the mechanism by which it transfers; e.g., vapor phase transfer, displacement, etc. This information may allow us to develop methodology for increasing the amount transferred from the filter which could result in more efficient utilization of certain flavorants.

I. Synthesis and Evaluation of New Flavorants

We will continue this ongoing project with most of the emphasis being placed on the synthesis of tobacco identical terpenoids.

J. Structure-Activity Relationship for Flavorants

This is a long-term project which has as its ultimate goal the development of an understanding

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of the relationship between a substance's structure and its flavor so that new flavorants can be prepared on a rational basis. The first stage of this program must be the establishment of non-subjective descriptors of flavor. We are presently investigating the use of multi-dimensional scaling techniques to accomplish this goal.

K. Nicotine Research

Our primary goals for this project continue to be the synthesis of a nicotine analogue which shows separation of effects and to elucidate the molecular mechanisms by which nicotine exerts its biological effects.

L. Service to Other Groups

A more detailed explanation of these programs is provided in the individual projects' plans and objectives which are attached.

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: E. B. Sanders

Date: October 30, 1981

From: R. A. Kornfeld

Subject: Plans and Objectives for the Chemical Research Instrumentation Section for 1982.

This memo outlines the plans and objectives of the Chemical Research Instrumentation Section for 1982. The responsibilities of the section are to:

1. Provide an analytical resource for collaborative research and service requests in the areas of mass spectrometry, gas chromatography/mass spectrometry, pyrolysis, and laboratory data acquisition.

2. To ensure that future needs are met by improvements to currently available equipment and methodology as well as the purchase of new equipment.

In addition to handling the submission of samples on an as needed basis, several ongoing programs will be using the facilities:

1. Materials Evaluation (ongoing)

Materials that come in contact with cigarette components have been screened for the production of potentially undesirable compounds by using pyrolytic techniques. If initial screening methods are inconclusive, the sample is subjected to pyrolysis/GC/MS analysis. These analyses will continue in 1982.

2. Flavor and Flavor-Release Screening (ongoing)

As part of the screening protocol for flavor additives to tobacco, candidate compounds are subjected to pyrolysis/GC/MS analysis so that toxicity of any thermal decomposition products can be assessed. Volatile products will be identified using equipment in the section.

3. Aza-arenes from Nicotine Decomposition

The principal function for mass spectrometry in this project is the identification of selected aza-arenes in cigarette smoke. This endeavor will involve reproducing chromatographic patterns obtained by other investigators, obtaining GC/MS profiles, identifying candidate aza-arenes, and determining if other compounds are coeluting with these aza-arenes.

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Plans for instrument modification are as follows:

1. Uses for DuPont GC/MS System

Because of the limitations in its scan speed, the DuPont GC/MS system will be used only for requests involving packed column analysis. The major uses for this system are forecast to be:

a. the identification and structure confirmation of synthesized compounds.

b. the on-line analysis of pyrolytic products from routine requests when the DuPont has pyrolysis capability (1st Quarter).

c. other analyses that can be satisfied using packed columns.

There will be a pilot program started in 1982 that will involve the use of the DuPont system by trained individuals other than section personnel. The training program will involve identifying individuals interested in using the DuPont GC/MS, training these scientists, monitoring their progress, and then allowing them to use the system. Of course, parts of the system that are easily susceptable to damage will be handled by section personnel. After the system is running well, the operator training program will begin (2nd Quarter).

2. Uses for the Finnigan GC/MS System

The principal functions for the Finnigan GC/MS systems are:

a. to process requests requiring the use of capillary GC techniques.

b. to participate in projects that require more complicated analysis than would be handled using the DuPont system.

Both of these responsibilities relate to the Finnigan system being used more in a research function. There are several modifications that will increase the capability of this instrument.

First change involves replacement of the data system to allow for both faster processing of spectra and greater capability

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for computerized file search of reference spectra. Additionally, the updated computer allows for simultaneous collection of data and processing of information from a separate data file. With the approval of the capital expenditure in 1981, this system will be functional by 2nd Quarter, 1982.

A second project is the installation of pyrolysis/capillary GC capability on the Finnigan system. The coupling of pyrolysis equipment with capillary columns will provide a powerful tool for studying the thermal decomposition of systems such as cooked flavors, Amadori compounds, aza-arene precursors, and flavored tobacco. This modification will be completed by the third quarter.

The other modification to be undertaken in 1982 is the insertion of a split between the GC and MS. The split will result in the ability to detect a minor component coeluting with a major one. Two specific detectors that could be attached to the split are a nitrogen selective detector or a radioactivity detector. The former detector would be useful for studies involving cooked flavors, nitrogen-containing flavors in smoke, and aza-arenes. The latter detector would serve to provide direct confirmation of the identity of radiolabelled material instead of relying on an unchanging chromatographic pattern from radio GC to GC/MS. This modification will be completed by the 3rd quarter.

Plans for laboratory data acquisition for the Chemical Research Division involve discussions about the type of data collection network, the specific needs of the various projects, and the type of system to purchase. These subjects will be discussed in conjunction with the search for a scientist to head the acquisition and installation of such a system.

Rich Kornfeld

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: E. B. Sanders Date: October 28, 1981

From: J. I. Seeman

Subject: Plans and Objectives for 1982 (Charge #2500)
(Chavdarian, Clawson, Secor, Seeman, Southwick, one co-op)

Work for 1982 will be focused in two general areas; alkaloid (nicotine) chemistry and flavor chemistry. In addition, we will continue to perform assistance to other units upon request in such areas as custom synthesis and general organic chemistry.

I. ALKALOID AND NICOTINE CHEMISTRY

A. Objectives

1. To develop a fundamental understanding of mechanisms by which nicotine and other tobacco alkaloids interact with peripheral and central nervous system receptors in vitro.

2. To determine if nicotine analogues can be designed which exhibit differential in vivo activity, in particular, to separate out central nervous system responses from peripheral responses.

3. To determine if nicotine analogues can be designed which exhibit differential activity at different receptors in vitro.

4. To assist the Behaviorial Research group's efforts to delineate central and peripheral nicotinic effects; to examine the nature of reinforcement, effect on on-going behavior, etc.

5. To establish PM's state-of-the-art participation world-wide in nicotine research so that PM's results can impact on external research.

6. To develop correlations between in vitro systems (e.g., receptor binding) and in vivo tests (e.g., behavioral studies and rat blood pressure).

7. To investigate the possible correlation of structural and chemical parameters with biological behavior.

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8. To develop procedures to synthesize nicotine analogues and isotopically labelled nicotine analogues.

9. To perform, in a collaborative fashion, pharmacological testing of nicotine and its analogues with a goal of deriving structure-activity relationships.

10. To develop an effective insecticide(s) through collaborative testing of nicotine analogues; in this conjunction, the mode of action(s) of these compounds will be investigated.

11. To aid other groups with problems related to tobacco alkaloids (e.g., to develop procedures for nicotine recovery from various manufacturing residues and effluents).

B. Synthetic Studies

1. Preparation of Radiolabeled Nicotinoids

An essential aspect of nicotine research involves the isolation and identification of nicotinic receptors. Related to this goal is the determination of nicotine distribution in mammalian brain. Radiolabeled nicotinoids are required to achieve these goals. We will develop procedures to prepare optically pure (S)-nicotine of high (10-20 Ci $mmol^{-1}$) specific activity as well as routes to analogues possessing lower levels of activity. The most direct routes to date include (1) the reduction of N'-methyl-myosmine(s), and of 5'-cyanonicotine; and (2) the N-methylation of nornicotines.

2. Preparation of Affinity Chromatographic Substrates

The most modern of procedures for the isolation of nicotine receptors involves affinity chromatography. We will continue our current efforts in collaboration with Prof. Leo Abood by preparing additional quantities and amounts of ligands for this work. Prime to this target are hydroxynicotinoids.

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3. Preparation of Nicotinic Receptor Irreversible Binding Agents

These materials can have significant use in a variety of novel fashions. For example, we may be able to develop a long-lasting CNS nicotinic agent, as judged by prolonged behaviorial responses in Vic DeNoble's behavioral tasks. These will allow the identification of CNS sites of action. Candidate compounds for this will be derived from the hydroxynicotines discussed in item 2 above, most probably halogenated nicotinoids.

4. Preparation of Analogues for Pharmacological Testing

All of the substances listed above will be examined for a wide range of pharmacological properties, along with other compounds prepared specifically as part of our goal of defining nicotine's structure-activity profile and developing agents having specific activities.

A major effort will continue toward the preparation of optically pure nicotinoids. We will also direct our attention on 6-substituted nicotinoids, acyclic analogues, bridged nicotines, heterosubstituted nicotines, and specific compounds such as 2-methyl-3,3'-isonicotine and 6,2'-dimethyl-nicotine. We will follow leads developed from our blood pressure and behavioral testing program, etc.

5. Development of Procedures for the Purification of Nicotine Analogues

As part of the synthetic efforts listed above, an important and difficult task involves the purification of the analogues. Techniques will be developed which utilize HPLC and thick layer/Chromatotron chromatography.

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II. FLAVOR CHEMISTRY

A. Objectives

1. To evaluate a broad base of tobacco flavor types.
2. To develop novel flavor systems.
3. To develop a patent position in tobacco flavorants.

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4. To develop structure-activity relationships in flavor systems which have importance in the tobacco field.

5. To develop and maintain an expertise in areas known to be important to tobacco flavor.

6. To assist Flavor Development.

7. To develop an understanding of how flavorants can contribute to low tar systems.

8. To follow-up flavor leads developed by other PM areas.

9. To initiate work which could have impact with respect to flavors and commercial products.

B. Plans

1. An odor profiling system will be developed to begin to obtain internally consistent data on a broad range of odorants and flavorants. This data base, with suitable analysis, will be useful to suggest new areas of flavor research based on the perceived odors. The steps involved in setting up this program are:

   a. select reference odorants from flavor library
   b. select 5-8 flavor personnel to do ratings
   c. prepare ballots (based on Dravnieks scheme)
   d. arrange data analysis with Danny Ennis

2. Vigorous efforts will be devoted to investigating structure-property relations in olfaction and flavors. During the past year a thorough evaluation of techniques of structure-activity research was conducted and summarized as a working memo to suggest a direction to pursue. This subject area represents a long term program designed to guide the development of new flavorants. During 1982 the goals are:

   a. refine multidimensional scaling (MDS) techniques - evaluate effect of concentration and choice of compounds on relationships among compounds

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b. implement pattern recognition capability to analyze data from odor profile study

c. implement molecular property calculations to correlate odor properties

3. Extend acylation/alkylation program to include thiazoles and pyrimidines. The products obtained will expand our patent coverage of the acylation process developed during 1981 and also serve as potential flavorants and odorants for MDS studies. The thiazoles are of interest because they have an interesting stereoelectronic similarity to pyridines.

4. The components isolated from the abienol work will be further investigated in order to establish their identity unequivocally. In particular the components of interest have a dusty-woody odor which is perceptible in mainstream smoke.

5. An amicable relationship has been developed with Flavor Development during the past year. Early in 1982, Flavor Development will occupy new quarters in the Technical Center. This increased physical separation will require a special effort on my part to continue close cooperation. To ensure this I propose the following actions:

a. formalize panel meeting times for evaluation of Phase I submissions
b. develop a panel for evaluating Phase II submissions thoroughly
c. incorporate Chemical Research presentations into monthly Development Meetings

These actions will further cooperation and improve the process of evaluating potential flavorants prepared in Chemical Research.

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III. Development of Structure-Activity Relationships in Nicotine Pharmacology and Olfaction. The Use of Chemical Reaction Modeling for Biological Profile Correlations.

An important aspect of our work during the last

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few years has been the derivation of structural and reactivity information in the nicotine field. This information has allowed us to better understand the pharmacological properties of the nicotine analogues and to predict interesting compounds to prepare. In 1981, we have begun to extend these concepts to our interest in olfaction structure-response relationships. We have begun a very productive collaboration with Professors John Schug and Jimmy Viers of the Virginia Polytechnic Institute and State University involving theoretical aspects of pyridine, pyrazine, and nicotine structure, reactivity, and electronic distributions. We intend to implement molecular property calculations so as to correlate odor properties of series of compounds.

A. Mechanistic Studies

1. Kinetic experiments and stereochemical evaluations of the alkylation of a wide variety of nicotinoids with iodomethane and possibly other alkylating agents will continue. These experiments are aimed to allow an understanding of the steric, electronic, stereoelectronic, and conformational features present in these systems. A totally automated conductivity system has been implemented and will facilitate the ease of these studies.

B. Protonation studies will continue to allow the evaluation of the conformation of the N-methyl group in these nicotine analogues.

C. NMR studies will be used as in the past to derive conformational information about these molecules. Barriers to rotation for a number of analogues will be determined.

D. Theoretical calculations (INDO, Ab Initio) will be performed to give information regarding conformation, electron distribution, polarizability, etc., of these molecules.

E. Kinetic studies involving α-cyanoamines will be performed.

cc: V. DeNoble
D. Ennis
Members of 2500

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: E. B. Sanders Date: November 2, 1981

From: R. A. Comes

Subject: Plans and Objectives for 1982 (Charge #2501)

Charge #2501 has as its basic objective the investigation of smoke chemistry utilizing radiochemical techniques. Programs which will be ongoing during 1982 can be categorized briefly as follows:

1. Low Level Laboratory - Completion of the low level laboratory, including equipping of the facility, will occur in 1982. This facility will ultimately perform both service and basic research functions, though it is anticipated that only service work, namely environmental monitoring of various tobacco samples, will be initiated in 1982. Related to the low level laboratory is the project's work in health physics.

2. Precursor-Product Studies - The use of radiolabelled precursors to enable one to determine products formed on a burning cigarette, thereby developing an understanding of the smoke chemistry of the selected precursor, is the major role of Charge Number 2501. A number of specific studies of this type will be carried out in 1982 including investigations of the formation of carbon monoxide from labelled cigarette paper, the behavior of menthol on a labelled cigarette, formation of aza-arenes from nicotine, and the formation of volatile nitrosamines from nitrate and/or nitrite.

3. Transfer Of Inorganics To Smoke - This work constitutes a long term study regarding the mechanism of transfer of non-volatile substances to mainstream smoke.

4. Sidestream Smoke - Methods will be developed for the collection and analysis of sidestream smoke.

More detailed descriptions of the specific objectives for 1982 are given below.

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Source: https://www.industrydocuments.ucsf.edu/docs/nhml0000

I. Low Level Laboratory and Health Physics

A. Low Level Laboratory

1. Finish construction of the low level laboratory to include:

a. overseeing all aspects of construction leading to and including final building acceptance and occupance

b. evaluation, ordering and installing of necessary counting equipment in conjunction with outside consultants

2. Place purchased and on-hand counting equipment into routine operation to include:

a. set-up and check-out of all equipment to insure proper operation-calibration standards required

b. establishment of normal background radioactivity levels on natural samples (air, soil, water)

c. establishment of techniques for determination of radioactivity levels in our products, raw materials, water supplies and effluents, etc.

3. Initiate a baseline radiochemical study for annual monitoring of tobacco crops in order to be able to measure year-to-year variability of natural radioactivity levels and thus be able to respond to changes as may be caused by natural and/or man-made disasters

B. Health Physics and Administration

1. Maintain all aspects of health physics for the Research Center to include:

a. routine inspections of all areas of radiochemical useage to insure proper storage, handling and disposal of radioactive materials

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b. training of personnel to insure their health and safety during all radiochemical activities

c. assurance of proper disposal of radioactive waste and its subsequent removal from the R&D area

2. Administer requirements imposed by virtue of possession of an NRC license to include:

a. proper record keeping as required for health physics

b. maintenance of a library of pertinent and required texts, publications, etc.

c. continuous updating of information to insure compliance with all new NRC restrictions

II. Precursor-Product Studies

A. ^{14}C-Menthol Study

Cigarettes have been prepared to which ^{14}C(U)-menthol has been added. The cigarettes have been stored in individual vials and are currently being monitored for equilibration and transfer of menthol from filler to filter. Studies to determine the effects of different paper porosity, varied levels of dilution and varied total cigarette length on the menthol distribution and breakdown in smoke are to be conducted. In addition, the rate and final migration data from filler to filter will be determined. Comparisons of all data will be made with previously determined values from non-diluted, unfiltered ^{14}C-menthol cigarettes.

B. Aza-Arenes

A study to determine if nicotine is a precursor to the aza-arenes known to exist in cigarette smoke is to be conducted. Several memos detailing the major goals of the study and other means to accomplish these goals have been written. In particular the immediate directions to be followed require:

1. close co-operation between several projects on all aspects of the work

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2. ^{14}C-tobacco plant production and isolation of $^{14}C(U)$-nicotine from these plants

3. incorporation of highly purified $^{14}C(U)$-nicotine into specified cigarettes, smoking of these cigarettes and isolation of the aza-arene fraction(s) from the WSC

4. receipt, installation, check-out and methods' development of capillary gas chromatographic instrumentation and techniques

5. development of methods for the sophisticated handling (heart-cutting, GLRC, splitting) of fractions containing potential ^{14}C-labelled aza-arenes

C. ^{14}C-Labelled Cigarette Paper

Cigarette papers have been prepared by Schweitzer Paper Company with and without citrate added and incorporating ^{14}C-cellulose isolated from ^{14}C-labelled stalk and stem from $^{14}CO_2$ chamber grown tobacco. A study is to be conducted utilizing cigarettes made from these papers to investigate the effect of citrate on cellulose breakdown into smoke-particular emphasis is to be placed on the contribution to CO formation. Future plans include an extension of this study using papers prepared from ^{14}C-citrate, $Ca^{14}CO_3$ and $^{45}CaCO_3$ and the determination of the contribution of each to the various smoke phases.

D. Puff-by-Puff Studies

Continue studies presently underway to investigate similarities and differences in the deliveries of various components within a puff and between selected puffs. Studies using cigarettes labelled independently with ^{14}C-bright, -burley, and -Oriental tobaccos are being conducted to determine their contribution to the puff profiles.

E. Low Delivery Cigarettes

Continue studies underway in which ^{14}C-bright, -burley, and -Oriental tobaccos are incorporated into selected low and ultra low tar cigarettes. Investigations center on delivery from the major cigarette components into smoke and the relation of the percent delivery to the original percentage in the cigarette: i.e., does each major component deliver its proportional share to the mainstream smoke?

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F. Volatile Nitrosamines

1. In conjunction with the aza-arene study, labelled nicotine and/or other nitrogen containing natural tobacco constituents will be isolated and utilized to determine their contribution to the volatile nitrosamines in cigarette smoke.

2. Methodology will be developed to interface the exit of a thermal energy analyzer with a mass spectrometer so that the ratio of $^{14}NO_2$ to $^{15}NO_2$ formed from the pyrolysis/oxidation of a nitrosamine can be determined.

3. Studies will be carried out using added $^{15}NO_3^-$ and $^{15}NO_2^-$ to various types of cigarettes to attempt to elucidate the mechanism of volatile nitrosamine formation.

III. Calcium -45 Studies

Conduct a series of studies utilizing tobacco grown hydroponically in nutrient solution containing $^{45}CaCl_2$ to include:

a) total smoke distribution of calcium - in particular the previously observed negligible delivery of calcium into mainstream smoke will be investigated as will the calcium contribution to the sidestream smoke

b) determination of the filtration of calcium by the tobacco rod and by filters during smoking and comparison of this data with previously determined values for other inorganics

c) assistance to the microstructure group in their studies on calcium location in leaf before and after curing and the effects of expansion on calcium distribution

IV. Sidestream Collection and Analysis

Initiate a program to effectively collect and analyze sidestream smoke (defined as that smoke generated in the 58 second interval between mainstream puffs). Some technology is available for individual cigarette SS collection from non-radioactive cigarettes but techniques for radioactive total trapping must be developed. The trapping of SS smoke from large

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numbers of cigarettes presents unique problems that must be addressed, particularly as to the handling of the mass of water generated and the safe treatment of the radioactivity in the smoke. The simultaneous collection of MS and SS is the third major area to be investigated in this program. Each component of the three areas requires the development of special techniques for trapping and handling. The ultimate long-range goal is to generate smoke data comparable to that reported by tobacco researchers in the past on mainstream cigarette smoke.

V. Miscellaneous

1. Continue studies directed at the incorporation of radioactive counting equipment onto capillary gas chromatographs.

2. Perform routine and non-routine radiochemical determinations for this and other projects and respond to other non-radiochemical inquiries as requested.

Roger Clowes

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: E. B. Sanders

Date: October 28, 1981

From: R. T. Bass

Subject: Plans and Objectives for 1982
(Project #2506 - R. Bass, G. Newell, and A. Brna)

1. To provide ^{14}C radiolabelled tobacco for investigations into the process by which cigarette smoke is formed and delivered; also to grow tobacco plants with selected isotopes (i.e. ^{45}Ca, ^{32}P, ^{35}S), to cure the harvested leaf, and to fabricate cigarettes for determinations of the fate of these isotopes in smoke. We have produced ^{45}Ca tobacco which will be utilized to follow the fate of ^{45}Ca in cigarette combustion.

2. To grow and provide fresh green tobacco plant materials in order to supply various samples as requested by other R&D Projects; also to provide and maintain mature tobacco plants for various display purposes at R&D and at the Manufacturing Center.

3. To continue to investigate the growing and use of N. rustica to provide a source of ^{14}C labelled minor alkaloids to be used in smoke studies.

4. To blend, cut, spray, and process various tobaccos and fillers as required and to subsequently prepare special hand made cigarettes for smoke studies.

5. To provide and maintain an inventory of ^{14}C labelled plant materials, and regular tobacco samples of various types and characteristics used in blend and cigarette production.

6.. To provide appropriate responses to various special inquiries and review certain manuscripts as requested by management.

7. To provide liaison with PM R&D, the Leaf Department, Tobacco Industry Committies, commerical companies, Federal and State Agricultural Research Agencies, and growers on a cooperative basis, to test and evaluate any necessary tobaccos or materials deemed to be in the best interest of the company.

8. To continue the production of ^{14}C tobacco in the Greenhouse chamber growth system specifically for the VDC nicotine work, as related to decomposition of nicotine during cigarette combustion and the subsequent precursor-product relationships.

9. To investigate and update our capabilities as related to the techniques of sheet making procedures used to incorporate

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^{14}C materials into a usable physical form for cigarette making and subsequent smoke studies (e.g., the fate of ^{14}C protein during combustion and its possible contribution to the formation of aza-arenes and nitrosomines, etc.).

Roger T. Bass

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Dr. E. B. Sanders

Date: October 27, 1981

From: Y. Houminer

Subject: Plans and Objectives of Project 2515 for 1982

FLAVOR-RELEASE CHEMISTRY

Work during 1982 will focus on both synthetic and pyrolytic aspects of different flavor release systems.

GENERAL OBJECTIVES

1. To develop release systems for flavors which cannot be added directly to tobacco because of either their high volatility or their very low odor threshold. To obtain patent position in this area.

2. To examine the relationships between structure and release efficiency in a variety of systems. To carry out systematic research in that direction so that structure-efficiency correlation can be obtained and used in the future for the design of flavor release compounds.

3. To use different release systems as models for understanding the mechanism of smoke formation. In particular we would like to understand the factors affecting sidestream and mainstream deliveries.

4. To provide assistance to development upon request, in connection with objective No. 1.

5. To carry out research in the area of cooked flavors, focusing mainly on the melanoidin fraction of the cooked flavor composition. To isolate, characterize and identify the components of this fraction and to study their pyrolytic behavior.

6. To continue carrying out basic research related to some new and important scientific discoveries made during our routine research in connection with the above objectives.

PLANS

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1. Sugar-based release systems (acid release).

Last year we had spent much of our time on the purification and structure determination of sucrose-tetraesters isolated from Oriental tobacco. Unfortunately, during the recent 1981 TCRC

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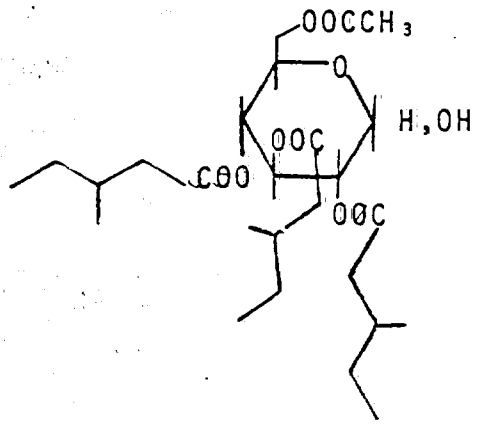
meeting researchers from the USDA reported the isolation of the same product. We are now waiting to receive more detail from the USDA people about their work. Nevertheless, we will make an effort to synthesize at least one of the sucrose tetraesters. We will first develop synthetic routes to D-glucose tetraester I and then study the feasibility of coupling it with a suitably protected D-fructose. We will also explore the possibility of preparing other disaceharides derived from I.



I

We will continue our synthetic efforts in preparing positional isomers of glucose mono esters. We will also try to increase the number of esters per glucose unit. This series of compounds will be pyrolyzed and is also targeted to meet goals given in objectives 1 and 2. We also plan to explore the use of partially acylated starch as an acid release agent. (Continuing 4th Quarter).

2. Sugar-based release systems (phenol release).

Our initial work in the area of disaceharide glycosides appears to be very promising. We will optimize synthetic procedures and extend them to a variety of phenolic flavorants. We will carry out smoke studies to demonstrate the advantage of disaccharides as carriers. These systems may turn out to be useful for MS/SS delivery ratio studies. (3rd Quarter).

3. Sugar-based release systems (thiol release).

In the past we have looked at ethylthioglueoside as a potential release precursor for ethanethiol. We plan to further explore this area by preparing a number of thioglycosides of disaccharides and study their pyrolytic behavior (3rd Quarter).

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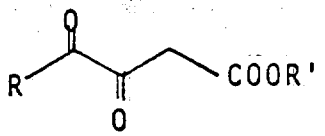
4. Ketone and diketone release.

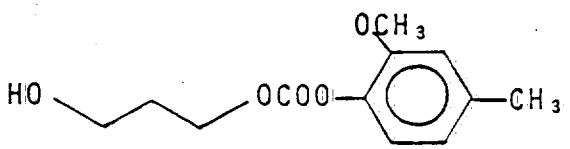
We have nearly completed our work in the area of β-ketoesters derived from sugars. The material will be summarized and submitted as a patent disclosure (1st Quarter). We plan to extend this research to 1,2-diketones, such as biacetyl, by preparing release systems having the general structure II, where R' is a suitable carrier such as glucose, glycerol etc. (2nd Quarter)



II

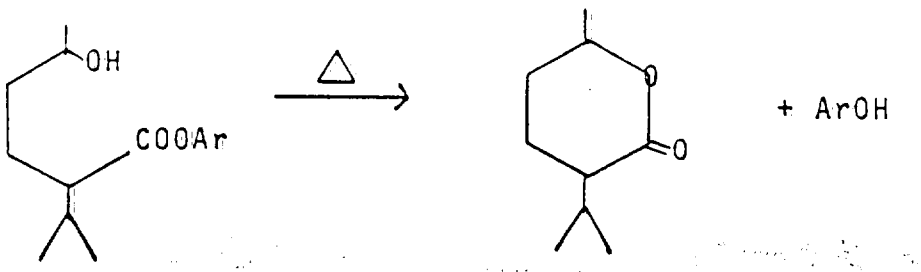
5. Phenol-release from low molecular weight precursors.

We have recently concluded that glycerol does not serve as a good carrier for the release of phenols from its carbonates. However, the monocarbonate derived from 1,3-propanediol (III) appears to be a more promising release agent. We will prepare a few analogues of III and study their pyrolysis. We also plan to use 5-hydroxyesters, such as IV, for the release



III

of phenols. In the following example the resulting lactone is a tobacco identical compound (3rd Quarter).



IV

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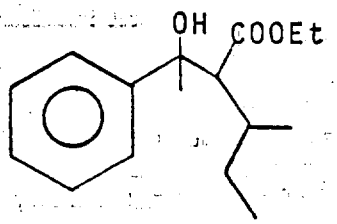
6. Glycerol-based acid release systems.

We have started to explore this area during the past year and plan to continue in the same direction. In particular we will evaluate the mono-esters that have already been prepared. We plan also to test glyceraldehyde as a carrier for acids. We expect to observe differences for glyceraldehyde esters as compared to glycerol esters. (1st Quarter).

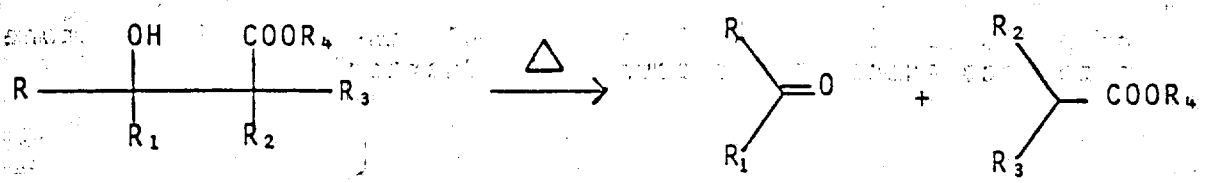
7. Ketone and ester release from β-hydroxyesters.

During the past year we have prepared a very good release system for ethyl 3-methylvalerate. This system (V) which is a β-hydroxyester, is now being tested by Flavor Development. We plan to supply Development with a large quantity of V upon request.



V

We plan to further explore the pyrolytic behavior of a large number of β-hydroxyesters (VI) where we hope to be able to establish a correlation between structure and release efficiency. We will focus on steric and electronic effects both on the α- and β-positions of the propionate skelton. This research may also help us in meeting some of the goals stated in



VI

general objectives 2 and 3. We will also explore the use of these systems for the release of ketones by condensing them with high molecular weight esters that have no significant flavor properties (continuing 4th Quarter).

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Source: https://www.industrydocuments.ucsf.edu/docs/nhml0000

8. Cooked flavors.

We have recently started to work in this area. We plan to develop methods that will enable us to isolate the melanoidin fraction from the cooked flavor mixture. We will try to characterize these compounds and in particular focus on their pyrolytic behavior (2nd Quarter).

9. Polymeric release agents.

In the past few years a large number of polymeric release systems have been prepared. During the coming year we plan to evaluate some of these polymeric precursors. (2nd Quarter).

10. Basic research - N.M.R. studies.

We plan to continue our study in the area of long range coupling through 4σ-bonds. To the best of our knowledge we are the first to observe such a coupling between a CH_3 and OH group in a none rigid system. We plan to publish these results next year (1st Quarter).

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: E. B. Sanders Date: October 27, 1981

From: W. B. Edwards, III

Subject: 1982 Plans and Objectives for Synthesis of Flavorants (Charge #2520)

With rising consumer demands for lower delivery cigarettes with excellent taste, the need for better flavorants is of increasing importance. The objectives of Project 2520 have been set to address this need for better flavorants and are:

1. To evaluate a broad base of tobacco flavor constituents.

2. To develop flavorants not found naturally in tobacco.

3. To obtain a solid patent position in tobacco flavorants.

4. To assist and support Flavor Development.

5. To develop and maintain an expertise in areas of importance to tobacco flavoring.

6. To initiate research which could have impact with respect to tobacco flavors and products.

Within commercial tobaccos (Bright, Burley and Oriental) the majority of the flavor constituents are composed of norisoprenoids derived virtually exclusively from diterpenoids (thunberganoids and labdanoids) and triterpenoids (carotenoids). As a consequence, our flavor efforts will be directed primarily towards the synthesis and evaluation of these types of tobacco constituents. If appropriate, analogs will be prepared to explore flavor potential or to support patent applications. In addition, compounds which are anticipated to be interesting flavors but have no counterpart in nature will be examined.

During 1982 the main efforts of the project will be directed into the following areas of research:

I. Terpenoid Flavorants (60%)

II. Heteroaromatic Flavorants (15%)

III. Flavor Development Support (15%)

IV. Cigarette Beetle Pheromone (10%)

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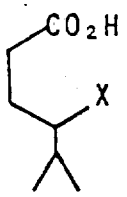
I. Terpenoid Flavorants

A. Thunberganoids and analogs. The major efforts of the project to date have been and will continue to be directed toward the synthesis and evaluation of thunberganoid derived tobacco constituents. During the coming year, we will be examining compounds of the following general types (I-V).



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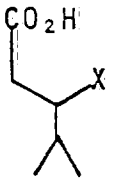

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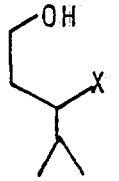

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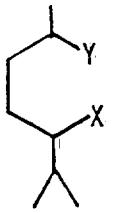

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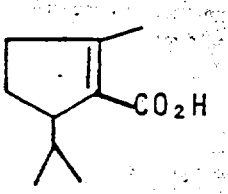


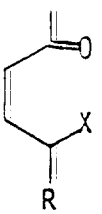
V

As results from flavor evaluation of our compounds become available, effort may be switched from the synthesis of tobacco constituents to either of the following areas.

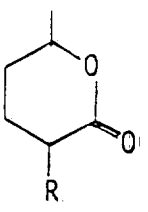
1. Analogs of a particularly interesting compound.

2. Scale-up synthesis for further evaluation.

Flavor evaluations have already indicated that compounds of the general types VI and VII perform well as tobacco flavorants. This area will be examined in further detail to explore the flavor properties of these groups and to establish a patent position.



VI



VII

R=alkyl and X=CHO, CO_2H, CO_2R

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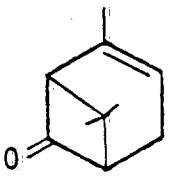
Source: https://www.industrydocuments.ucsf.edu/docs/nhml0000

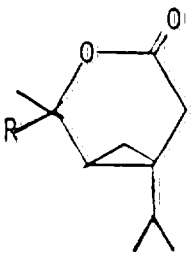
B. Carotenoids and analogs. Initial studies in this area will involve exploration of the chemistry of chrysanthenone (VIII). Opening of the cyclobutanone ring of VIII will give us access to a wide variety of carotenoid related 1,1,3-trimethyl-cyclohexanes. The current availability of verbenone, the precursor of VIII, and the limited research in this area, make this study particularly attractive.



VIII

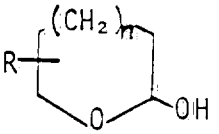
Concurrent with these initial studies, a detailed literature search on the carotenoid derived tobacco constituents will be carried out. Assimilation and correlation of the pertinent literature will be begun in order to establish priorities and direction.

C. Terpenoids. During the course of our research in 1981, a number of potential flavorant classes (IX-XI) have been discovered or proposed. At this time only limited work is planned with these compounds. This will be confined to the preparation of a few examples and their flavor evaluation. Based on the material's flavor attributes, further work may be done.



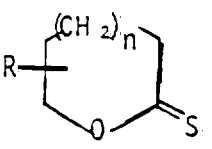
IX

Thujaketonic acid, lactones



X

lactols



XI

thiolactones

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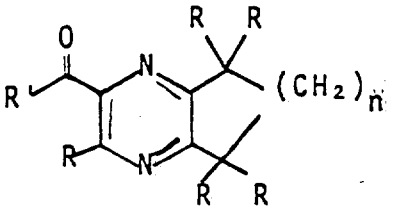
D. Flavorant Literature and File. Our efforts to collect the current, relevant flavor literature will continue. Concurrent with these efforts, work will continue on the development of a detailed file on terpenoid flavorants. If the necessary computer software is available, a retrieval basis for this file will be developed. Our final goal is to make accessible to the key personnel in flavor R&D all of the pertinent data on terpenoid flavorants.

II. Heteroaromatic Flavorants

A. Acylpyrazines. Work in this area will involve further use and development of the free radical induced acylation of pyrazines. Three projects are currently planned.

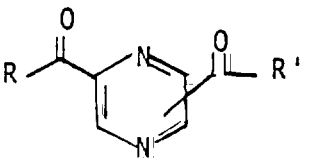
1. Synthesis of acylated pyrazines containing a fused alicyclic ring (XII).



XII

R'=alkyl R=H or alkyl

2. Preparation of mixed diacyl pyrazines (XIII).



XIII

R=alkyl R'≠ R = alkyl

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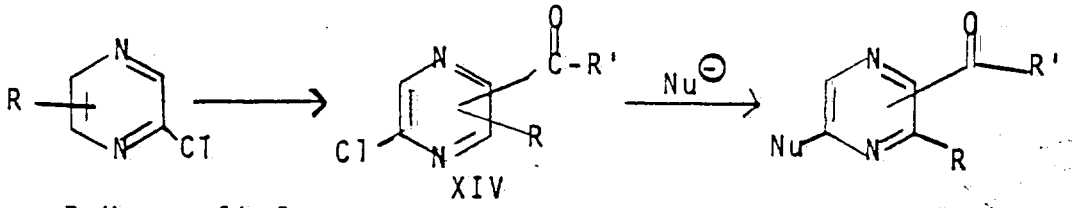
3. Synthesis of further chloroacylpyrazines (XIV), followed by displacement of the chloride by various nucleophiles.



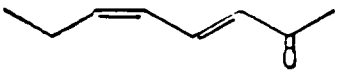
XIV

R=H or alkyl
R'=alkyl

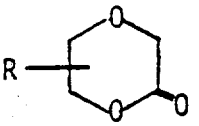
The first project will be started in the first quarter of 1982. With its completion, the other projects will begin in turn.

III. Flavor Development Support

The amount of effort to be placed in this area is difficult to estimate as it is dependent upon requests from Flavor Development. Currently, in the planning stages are the synthesis of a dieneketone (XV) and an evaluation of the literature on oxo-1,4-dioxanes (XVI) for potential flavor properties.



XV



XVI

IV. Cigarette Beetle Pheromone.

Studies on the cigarette beetle pheromone are being carried out in collaboration with Steve Long of Project 1101. The bulk of our contribution to this project should be completed during the third quarter of 1982. Our efforts will be directed towards exploration of an alternate pheromone synthesis and development of a separation of the serricornin diastereomers. As needed, additional quantities of pheromone will be prepared.

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: E. B. Sanders Date: October 30, 1981

From: H. J. Grubbs

Subject: Plans and Objectives (Project 2525)

General Objectives:

The general objective of Charge Number 2525 is to elucidate the chemistry which takes place on a cigarette during smoking. Programs have been developed which complement, rather than overlap those programs being pursued in Charge Number 2501. These programs and their objectives are:

1. Synthesis of radiolabelled, stable isotope labelled, or unlabelled materials for use in precursor-product relationships. These materials will be utilized to study thermal reactions which are likely to produce either undesirable or desirable products. Unlabelled compounds prepared to investigate the formation of desirable products will also be investigated directly.

2. Development and application of radioanalytical methodology to conduct precursor-product studies in situations where non-volatile products are formed. Such methodology will address the problems delineated above in uses where gas chromatographic techniques are not useful.

3. Isolation of specific substances from complex mixtures. Two purposes will be served by this endeavor. The first involves the isolation of radiolabelled natural products, obtained from chamber grown plants, to serve as starting materials in precursor-product studies. The second involves isolation and identification of products formed from model chemical reaction studies (see Item 4).

4. Smoke chemistry modelling studies. The end point of smoke precursor-product studies is the identification of a specific precursor for specific products. Such a study provides no information which would enable one to either increase or decrease (if not eliminate) certain products derived from a specific precursor except by total removal of the precursor which is rarely

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possible. We plan to initiate a program wherein the detailed chemistry of the product-precursor conversion will be studied in order to attempt to discover procedures which can be utilized to alter this conversion on a burning cigarette.

In order to accomplish these general objectives, the following specific projects will be pursued.

Aza-Arene Program

1. To delineate the alkaloid production of ^{14}C-labelled tobacco plants with regard to both quantitives and percentages of tobacco alkaloids produced. (Complete 1st Quarter)

2. To isolate and purify (to 99.99% purity) multi-gram quantities of ^{14}C-nicotine(U). (Complete 2nd Quarter)

3. To develop techniques to fractionate WSC for total removal of ^{14}C-nicotine(U) and simple (small) radiolabelled fragments of nicotine. (Complete 2nd Quarter)

4. To develop techniques for the isolation and radiochemical characterization of an aza-arene sub-fraction. (Complete 4th Quarter)

Natural Products Chemistry

1. To continue the isolation of ^{14}C-natural products from tobacco (or other suitable plant materials). Areas of interest are tobacco leaf glycosides, terpenes, structural carbohydrates, and amino acid/proteins. (Ongoing)

2. To develop alternate methods of obtaining ^{14}C-nicotine(U) with higher specific activity. A first approach is to explore the use of tobacco tissue culture (callus tissues). (Start 2nd Quarter)

3. To complete the isolation, identification, quantitation, and supply of samples of the anti-feedant azadiractin to Project 1101. (Complete 1st Quarter)

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4. To isolate and characterize additional quantities of rutin for product-precursor studies. (Complete 3rd Quarter)

5. To render technical assistance to outside projects when compatible with the accomplishment of our primary objectives. (Tonka bean extract, various botanical extracts for subjective evaluation, etc.) (Ongoing)

Synthetic Chemistry

1. To continue to prepare labelled materials for product-precursor studies within our project as well as satisfying other requests when compatible with current progress. (Ongoing)

2. To complete the preparation, isolation, and characterization of tobacco identical Amadori compounds for product-precursor studies (to be tested subjectively also). (Complete 2nd Quarter)

3. To complete efforts to obtain high specific activity specifically labelled menthol for use in non-destructive tracer work. (Complete 2nd Quarter)

4. To develop a preparation of specifically labelled cellulose for product-precursor studies. (Complete 4th Quarter)

Analytical Chemistry

1. To develop and optimize a suitable gas chromatographic method (probably heart-cutting) for the analysis of radiolabelled compounds (aza-arene, product-precursor studies demanding the ultimate detection limits). (Ongoing)

2. To develop a method for the study of the chemical composition of smoke aerosols. Both GC and HPLC techniques will be utilized. (Ongoing)

3. To develop HPLC methodology for the direct analysis of WSC and selective HPLC techniques for compounds by chemical class. (Ongoing)

4. To develop HPLC and GPC methodology for the study of the transfer of high molecular weight compounds to mainstream smoke (use of characterized polymeric materials). (Ongoing)

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5. To elucidate the mechanism of mainstream/ sidestream partitioning through the use of pyrolytic precursor-product techniques. (Ongoing)

Smoke Chemistry Modelling Studies

The high temperature interaction between secondary amines (or secondary amine precursors) and nitrate and/or nitrite will be studied under conditions which resemble the burning cigarette. Variations in conditions such as temperature, pH, catalysts, and surface conditions will be investigated. (4th Quarter)

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Dr. T. S. Osdene

Date: November 5, 1981

From: W. L. Dunn

Subject: Plans and Objectives - 1982

INTRODUCTORY REMARKS

Over the years the Behavioral Research Lab has stated its mission to be threefold: To study why, how and what cigarette smokers smoke. Of late we have been focusing more intensively on the question of why people smoke. Several reasons can be cited for this shift in emphasis: It is without doubt the most fundamental unanswered question for the cigarette industry today. As legal, political and social anti-industry pressures mount, the impetus for explaining smoking likewise mounts - to cause smokers' own behavior to be more credible to them, to strengthen legal defense arguments, to mollify legislators and to assist Philip Morris development people in product improvement. It is also true that the probability of being able to answer the question has increased as a result of new knowledge and new technology.

Smoking is complex behavior requiring any one or a combination of neuropharmacological and/or psycho-social explanations. We now believe that not only do smokers vary amongst themselves as to their motives for smoking, but a given smoker's motives will vary with psychological state and circumstance. The ultimate explanation is clearly not to be had in simple terms nor with a single conceptual model.

Our Behavioral Research program is therefore manned by psychologists with distinctive contributions to make; they represent a number of the many subdisciplines of the field, the subdisciplines selected because of their potential for contributing useful knowledge about smoking and the smoker. As an overview, the subdisciplines within the group and the personnel who represent them are presented here.

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Psychology Specialty	Researcher
Social Psychology	Sandra Dunn, Research Scientist
Electrophysiology	Frank Gullotta, Research Scientist Cindy Shultz, Assoc. Scientist A
Experimental Psychology I	Frank Ryan, Research Scientist
Experimental Psychology II	Jan Jones, Scientist
Behavioral Pharmacology	Vic DeNoble, Research Scientist Paul Mele, Scientist Yvonne Dragan, Assoc. Scientist A

Our plans and objectives are arranged hereafter by sub-discipline, accompanied by a brief descriptive statement which attempts to characterize the specialty.

SOCIAL PSYCHOLOGY PROGRAM

Nature of subdiscipline - Besides their more pragmatic work in public opinion and attitude surveys and market research, social psychologists have developed their own distinctive techniques and conceptual models to study the individual as a social creature. They have focused upon such phenomena as communication, conformity, role playing and the dynamics of the group process.

Objectives

1. To apply social psychology techniques to the study of cigarette preference.

2. To contribute to the understanding of those social forces which act as primary and/or secondary determinants of smoking behavior.

Planned Studies

The Direct Interview as a Means for Assessing Cigarette Preferences

The current state of the art of product testing still calls for continuing the search for more reliable, more sensitive techniques. We will be reviewing both individual and group interview formats with the intent to devise an alternative strategem for eliciting smoker reaction to new and improved products. Although this study is consigned to the social psychology program for planning purposes, the varied skills of

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the entire Behavioral Research Laboratory staff are "on call" for this effort.

The Pharmacological Versus the Psychological Effects of Smoking as a Stress Handling Strategem

Smokers do smoke more under stressful conditions. Is it the act of smoking as a behavioral event, or is it the pharmacological effects of components of smoke that mediate the inferred benefits derived from smoking? We are currently designing a series of studies that will be aimed at (1) partitioning out the respective contributions of these two driving forces, and (2) revealing their possible interaction.

Cigarette Smoking as Image Enhancing Behavior

It is generally agreed that the prospective smoker is attracted to smoking as part of the effort to confer adultness to the image of his person. These psychosocial values of smoking continue on to a greater or lesser degree with the regular smoker. Can we make a more definitive assessment of the characteristics attributed to smokers by both smokers and nonsmokers? Do these two groups differ in their social assessment of the smoker? If so, can we identify the critical attributed traits or characteristics? We will be designing a study this year to attempt to answer these questions.

Personality Correlates of Smoking

Previous research suggests that people may smoke for different physiological effects. Some smokers report that cigarette smoking has an arousal effect and others report that cigarette smoking has a calming effect. Based on findings from our research, we suspect that smokers who prefer low delivery brands may be those smokers who find cigarette smoking arousing and the smokers who prefer high delivery brands may be those smokers who find cigarette smoking calming. The basis for this hypothesis is that low delivery brand smokers seem to have these personality attributes that characterize people who seek to increase environmental stimulation - e.g. more emotional, anxious, etc. Similarly, the high delivery brand smokers evidence personality attributes indicative of people who are motivated to reduce stimulation from the environment - e.g. calm, peace of mind, etc. We plan to pursue these ideas, perhaps in the form of a laboratory study aimed at revealing these differences between the two groups of smokers.

ELECTROPHYSIOLOGY PROGRAM

Nature of Subdiscipline - Signals in the form of microvoltage changes in the electrical field at various points on the human scalp reflect events occurring within the underlying brain. At first encounter, the patterns are exceedingly complex and uninterpretable. Major technological advances, principally in

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the form of increasing analytical sophistication made possible by the application of the speed and multivariate capacity of the computer, are permitting the isolation of elements within the complex electrical pattern which represent heretofore unobservable neural events associated with the sensing and processing of stimuli.

Objectives

On the premise that the events which reinforce the smoking act are central nervous system events, we are systematically examining these newly observed electroneural signals for the effects of cigarette smoke inhalation.

Planned Studies

Olfactory Evoked Potentials

In March or April of next year we will begin experiments on olfactory evoked potentials (OEPs). The work will be done in collaboration with Flavor Development, specifically Dr. R. Ikeda. Our goal is to find means by which we can obtain useful information about certain subjective effects of tobacco flavorants in an objective, quantifiable and non-verbal manner. Dr. Ikeda has postulated that feeling factors, as mediated by free trigeminal nerve endings in the nose, are important to the pleasure one derives from smoking. We should be able to ascertain the degree of trigeminal involvement of different odorants by studying the waveform characteristics of the OEP. We hope to also categorize odorants in terms of OEP waveform morphology.

Perception and the Effects of Cigarette Smoking on Pattern Reversal Evoked Potentials

The purpose of this study is to assess the influences of both pharmacologic and perceptual factors on the pattern reversal evoked potential (PREP). We have previously demonstrated that cigarette smoking produces PREP latency shifts, and that these latency shifts reflect nicotine delivery. However, cigarette smoking also affects PREP amplitudes. Amplitude changes are frequently correlated with psychological state.

The study will elicit judgments by smokers of smoke intensity and record PREP measurements. The analysis is expected to permit the partitioning of (1) the effects of the absolute physical intensity and (2) the effects of the subjective perception upon the PREP measures. We expect to observe one kind of change in the PREP when a subject smokes a high delivery cigarette but perceives it low and a different kind of change if he perceives it high. We hypothesize that latency effects will reflect nicotine delivery, while amplitude effects will reflect the *perceived* intensity of what he has smoked.

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EEG Power Spectra in Cigarette Smoking and Abstinence

The purpose of this study is to systematically quantify the electrical activity of the brain under various smoking and deprivation conditions. Our interest in this area stems from both human and animal literature on the effects of smoking and nicotine on the EEG. There is a general lack of agreement on exactly what kinds of EEG alterations are produced by smoking and nicotine. Much of this disagreement could be resolved by quantifying the changes in a more systematic fashion. We propose to do this by studying EEG power spectra from different cortical loci under various deprivation and smoking conditions.

Smoke Deprivation and the Pattern Reversal Evoked Potential

We have previously conducted this study using flash evoked potentials. However, because flash EPs are so variable, both within and between subjects, our results were not easily interpretable.

Using pattern reversal evoked potentials (PREPs), we will conduct studies on individuals who are giving up smoking. We will bring them in for recording before they quit and then study them, over time, after cessation.

The question we are interested in is whether cessation alters the PREP and if it does, how long do these alterations last. We have reason to believe that quitting will distort the PREP for a time in some (but not all) individuals. We further believe that whatever PREP distortions occur will resolve over time.

EXPERIMENTAL PSYCHOLOGY I & II

Nature of Subdiscipline - This is the traditional research contingent of psychology. Sensation, perception, cognition and learning are the usual topics of study, with almost a century of laboratory experience embodied in methods, instruments and theory upon which to draw.

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Objectives (Experimental Psychology I)

1. To gain a better understanding of the role of nicotine in smoking

2. To study the basic dimensions of the cigarette as they relate to cigarette acceptability

Planned Studies

Salivary Nicotine

(This study appeared in the Plans for 1981. Subsequently Mr. Ryan was designated as the computer software person for the

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B.R.L., such that the time projected for this study was consumed in training and service in his new software specialist role. We anticipate completion of the study in 1982.)

It has been postulated that smokers modify smoking behavior to maintain certain levels of nicotine in the blood. Historically this has been the basis of nicotine titration hypotheses. Knowledgeable consideration of the issue suggests that the changes in level may be more important than the absolute levels - that the input of nicotine from a cigarette creates a "spike" which is the summation of the discrete puff-induced spikes.

We now have the ability to measure via gas chromatograph the level of nicotine in saliva. Observations from previous work with salivation and smoking suggest that systemic nicotine in saliva tracks with systemic nicotine in the blood. We plan to use the g.c. measure to:

A. Monitor the appearance and decline of nicotine in saliva following smoking. This will shed light on the question "Does a low systemic level of nicotine trigger the smoking response." The question can only be answered if measures are made many times. Therefore, we will:

B. Observe changes in salivary nicotine level across time and smokings, relating the changes to the delivery of cigarettes smoked and the time since prior smokings. The data will bear upon the issue to the extent that salivary nicotine reflects tissue and blood levels of nicotine. This must be confirmed by means of:

C. A correlational study of the salivary nicotine with blood nicotine. This is awkward research to perform because taking blood samples is intrusive and objectionable to participants and because it requires medical supervision. Obviously executing this segmant of the research will be contingent upon determining that there are systematic changes in the salivary nicotine data. We have made some preliminary contact with our medical staff, and they will support us when needed.

There are tentative plans for one other project in which nicotine will be delivered intravenously in different sized spikes of different duration, to yield a broader picture of the role of the spike, the level, and the reinforcement characteristics of the substance. The execution of this project is contingent upon the execution of study I-C above, since both involve the dosing of numerous subjects with nicotine.

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Smoking and Time Estimation

Time seems to pass more slowly in an unpleasant situation than in a pleasant one. In some of our research projects the

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participants have commented that the smoking sessions passed more quickly than the nonsmoking ones. If smoking does make time seem to pass more quickly, then we can understand why people smoke in boring or stressful situations (and perhaps why some people don't smoke on weekends but do smoke at work...!).

We will be conducting a series of studies in which smokers make some responses which are time related and some which are not to determine if these two classes of responses are differentially affected by smoking.

Objective (Experimental II)

1. To record and interpret changes in smoke inhalation patterns in response to changes in smoke composition.

2. To study the interrelationships of puffing, inhaling and nicotine retention.

Planned Studies

Changes in Smoke Inhalation Patterns as a Function of Nicotine Delivery

Data collection will continue into 1982 on this long-term switcher study. Because of the recent trend to low tar, low nicotine cigarettes the question has been raised whether the smoker is actually getting lower amounts of nicotine, or whether the individual is becoming a more efficient smoker by altering the intake behavior to compensate for reduced delivery. We are looking at changes in inhalation behavior as smokers switch among cigarettes delivering high (.94mg), medium (.5-.7mg) and low nicotine (.27mg) at constant tar deliveries (approx. 15mg).

Inhalation Patterns on Market Brands and their "Light" Versions

This study will continue into 1982, providing us with information on inhalation behavior as subjects smoke current market brands and switch to the "light" brand of the same name. In this study we are not isolating a single smoke component to experimentally manipulate, but rather are looking at the effect of the reduction in overall delivery. The inhalation data are being supplemented by puff profiles recorded on each brand at the end of the two-week switching period.

The Interrelationships of Puffing Dimensions, Inhalation Dimensions and Nicotine Retention

We are observing in our inhalation studies that inhalation patterns do change as a function of smoke composition. Are these changes "titration behavior", that is to say, adjustments toward maintaining some degree of constancy in nicotine intake, or are they caused by some other variable such as smoke acceptability?

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This year we will expand our inhalation program to address the following questions:

(1) Do puff parameters vary as a function of nicotine delivery?

(2) Do inhalation patterns vary with puff patterns?

(3) Do inhalation patterns influence nicotine retention?

(4) Which puff and inhalation parameters are the critical variables influencing nicotine retention?

The information will be obtained through the coordinated use of the smoking machine, the puff recorder, the Respitrace system (for inhalation measures), an exhalant nicotine collection device and a gas chromatograph. With this array, the fate of nicotine can be tracked from burning coal through successive intake events to exhalation into the ambient air.

BEHAVIORAL PHARMACOLOGY

Nature of Subdiscipline - This is the neuroscience branch of psychology, wherein psychologists, pharmacologists, physiologists and biochemists share a common conceptual model. Through observations of behavioral changes in response to the introduction of exogenous substances into the organism, inferences can be made about the substance, the organism and the effect of the former on the latter.

Objectives

1. To develop a better understanding of the actions of nicotine and other smoke compounds, especially those actions which reinforce the smoking act.

2. To empiracally differentiate nicotine from the classical abuse substances.

3. To use behavioral pharmacological methods to evaluate the nicotine-likeness of other compounds.

4. To contribute to knowledge of the neurological loci of the significant reinforcing event in smoking.

Planned Studies

Continue to investigate the reinforcing effects of smoke components.

Examine the nicotine analogues for reinforcing effects.

Examine the contribution of cholinergic, catecholaminergic,

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serotonergic and other neurotransmitter systems on smoke component self-administration.

Use discrimination and intraventricular administration techniques to develop structure/activity relationships among nicotine analogues.

Examine the effect of smoke components on frustrative non-reward behavior.

Begin collecting data on electrophysiological correlates of nicotine-induced behavioral changes.

Distinguish the pharmacological from behavioral effects of nicotine on schedule-controlled behavior.

Begin brain mapping studies to determine the sites of action of nicotine.

To further investigate the intraventricular self-administration of nicotine.

M.L.D.

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